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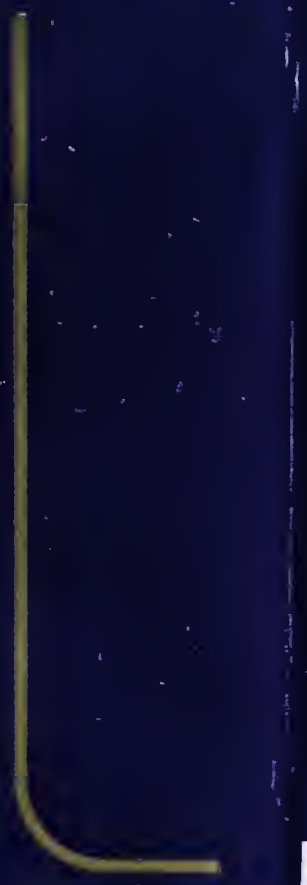
Industrial and Engineering CHEMISTRY



JANUARY 1946

ANALYTICAL EDITION

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INDUSTRIAL AND ENGINEERING CHEMISTRY
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Analytical Edition
WALTER J. MURPHY, EDITOR

Instrumentation in Analysis

In this issue we inaugurate a new monthly feature, Instrumentation in Analysis, prepared by Ralph H. Müller. Brief discussions of new instruments, methods, and apparatus of interest to analytical chemists, together with some discussion of their significance, field of application, and possible influence on the trend of future developments will appear over Dr. Müller's signature. It is recognized that many devices and methods, which are not primarily concerned with chemistry, will ultimately prove useful in analysis. This is particularly true in optical, electrical, electronic, and mechanical devices.

Dr. Müller is particularly well fitted by training and experience to cover the subject of Instrumentation in Analysis. He has been a member of the Radiation Laboratory of the Massachusetts Institute of Technology since 1942, and has done research in photochemistry, chemical physics, photoelectric photometry, electronics, instrumental methods of analysis and control, design of industrial and scientific instruments, and radar. He is well known to our readers as the author of comprehensive articles on photoelectric methods in analytical chemistry, American apparatus, instruments, and instrumentation, and instrumental methods of chemical analysis which appeared in 1939, 1940, and 1941. After receiving his Ph.D. from Columbia University and studying at Göttingen, he was on the staff of New York University from 1924 until he went to the MIT Radiation Laboratory to engage in highly secret research work for the war effort. In 1931 he received the silver medal of the American Medical Society for investigations on radium poisoning. Dr. Müller has been a member of the Advisory Board of the Analytical Edition for several years and extremely active in its deliberations.

It is felt by your editors that the introduction of Dr. Müller's column as a monthly feature is another step forward in a progressive and positive editorial program. The war stimulated greatly the development and use of instrumental methods of chemical analysis and unquestionably this trend will continue to grow in the future. There has been a long-felt need among analytical chemists for a timely, unbiased, and authoritative discussion and interpretation of new developments in the field of instrumentation.

Your editors and Dr. Müller will welcome suggestions and constructive criticisms from our readers. We also will welcome suggestions on other subjects of special interest to analytical chemists that might be developed into regular monthly features with treatment similar to Dr. Müller's presentation on instrumentation. We are here to serve the profession and the broad field of analytical chemistry.

Determination of Aromatics, Naphthenes, and Paraffins by Refractometric Methods

R. M. GOODING, N. G. ADAMS, AND H. T. RALL
Petroleum Experiment Station, Bureau of Mines, Bartlesville, Okla.

Methods are described for determining aromatics, naphthenes, and paraffins in virgin naphthas and condensates boiling below 320° F. Most individual hydrocarbons can be estimated to 243° F. Efficient fractionation is used to produce narrow-boiling-range fractions containing relatively few individual hydrocarbons. Densities and refractive indices for the mercury g line and the sodium D line, determined for each fraction at 20° C., give specific dispersions and refractivity intercepts used for analysis of the samples. Aromatics are determined from specific dispersions and naphthenes, and paraffins are determined by use of modified Kurtz and Headington refractivity intercept–density charts. Aromatics can be determined from these charts with some loss in accuracy. Development of the charts from experimental and literature data is discussed and illustrated. Analyses of ten crude-oil naphthas are given.

THE Bureau of Mines, since early in 1942, has been analyzing various virgin naphthas and condensates to determine their possibilities as sources of aviation-gasoline base stocks, toluene, and other hydrocarbons. These analyses are based essentially upon fractional distillation of the naphthas or condensates, and refractometric examination of the distilled fractions. The final data obtained from such analyses indicate the quantities of aromatics, naphthenes, and paraffins boiling below 160° C. (320° F.) in the sample. The quantities of most individual hydrocarbons boiling below 117.22° C. (243° F.) can be determined. This report describes and illustrates the method of analysis adopted, shows the application of the method to the analyses of several crude oils, and indicates some uses of the results.

A study of the methods used and proposed for the determination of aromatics in saturated hydrocarbon mixtures indicated that aromatics in a crude oil could be conveniently and accurately determined from the specific dispersions of small narrow-boiling-range fractions obtained by distillation of the naphtha portion of the crude oil. Among others, Dixmier (*2*), Ward and Fulweiler (*9*), and Grosse and Wackher (*4*) have used specific dispersion in hydrocarbon analysis. The present method in which the specific dispersion is a measure of the quantity of aromatic material in a naphtha fraction, when that fraction contains only aromatics, naphthenes, and paraffins, is essentially a modification of that of Grosse and Wackher. Such a determination is possible because specific dispersions for naphthenes and paraffins are nearly equal and constant throughout the gasoline range, whereas the specific dispersions for aromatics, although not constant, are substantially higher than the values for naphthenes and paraffins.

The general equation for specific dispersion of a liquid is

$$S = \frac{n_a - n_b}{\mathrm{d}} \times 10^4 \qquad (1)$$

where S is specific dispersion, n_a is the refractive index for light of wave length a, n_b is the refractive index for light of wave length b, d is the density, and 10^4 is a factor included for numerical convenience. All measurements of physical properties are made at one temperature, which for this work is 20° C. Although dispersion measurements of Grosse and Wackher were made for the F and C lines of hydrogen, the measurements used in this work are for the mercury g line and the sodium D line. The latter two lines are used because convenient and reliable mercury and sodium lamps are now commercially available and, since the value of the term $n_a - n_b$ is greater for these two lines than for the F and C lines of hydrogen, the accuracy of the method should be improved. A further modification of the method of Grosse and Wackher is in the use of very narrow-boiling-range fractions (usually less than 1° C.) rather than fractions having a boiling range of about 25° C. The determination of accurate values for the specific dispersions of pure aromatics, and of an average value for mixtures of naphthenes and paraffins, as well as the determination of aromatics in crude-oil naphthas, is discussed by Thorne, Murphy, and Ball (*8*).

The determination of the naphthene content of a sample is based on the use of the refractivity intercept, an empirical property originated by Kurtz and Ward (*6*). The present method is a modification of an analytical procedure proposed by Kurtz and Headington (*5*) using this property and applicable to mixtures containing both saturates and unsaturates.

The refractivity intercept is defined by the equation

$$R = n_{\mathrm{D}} - 0.5\mathrm{d} \qquad (2)$$

where R is the refractivity intercept, n_{D} is the refractive index for the sodium D line, and d is the density of the liquid, all measurements being made at 20° C. As described by Kurtz and Ward and as used by Kurtz and Headington, R is a constant for each hydrocarbon type or homologous series. If a sample contains only aromatics, naphthenes, and paraffins, the use of the refractivity intercept as an analytical tool depends upon the relationship, usually shown graphically, between the refractivity intercepts and densities of the sample and of the individual aromatics, naphthenes, and paraffins thought to be present. Four major modifications have been made in the method of Kurtz and Headington: (1) only aromatics and saturated hydrocarbons are considered in the scheme of analysis; (2) the graphical analysis charts cover an average boiling range of 10° C. instead of 30° C.; (3) very narrow-boiling-range fractions are analyzed; (4) individual values for refractivity intercepts are used for all compounds instead of a single value for each type.

After the aromatic and naphthene contents of a fraction containing only aromatics, naphthenes, and paraffins have been determined, the paraffin content of the fraction is obtained by difference. Aromatics can be determined by the use of refractivity intercept and density, but it is felt that the present determination of aromatics from specific dispersions is more accurate. A comparison of results by the two methods is given in Table I.

ANALYTICAL APPARATUS AND PROCEDURE

The accuracy of the analytical method described below depends in part on the apparatus, and its quality must be such that data of the requisite accuracy and precision are obtained. The equipment described in the following paragraphs has been found satisfactory for this work.

Two types of columns are used for the fractional distillation of samples. In one assembly the column proper is a glass tube 21 mm. in inside diameter packed with single-turn wire helices 2.4 mm. ($^3/_{32}$ inch) in diameter. The packed section is 244 cm. in length, and is insulated with asbestos listing and magnesia. A system of electrical heaters is incorporated in the insulation, so that the temperature of the insulation can be maintained at or slightly below the temperature of the vapor and liquid within the column. These temperatures are measured by 5 pairs of iron-constantan thermocouples spaced along the length of the column. The glass still pot is permanently sealed to the column proper and has a capacity of 4.5 liters. A total condenser at the top of the column provides reflux and liquid overhead product. The column was tested in the usual manner using methylcyclohexane and isooctane, and has an efficiency at total reflux equivalent to 88 theoretical plates.

The other distillation unit is a commercially available vacuum-jacketed glass column having 122 cm. of patented wire packing.

Table I. Comparison of Determination of Aromatics by Specific Dispersion and by Refractivity Intercept–Density Methods

Volume Per Cent of Aromatics in Naphtha or Condensate

Aromatic	Carthage			Chapel Hill			Conroe			East Texas			Hull-Silk-Sikes (Strawn)		
	Specific dispersion	Refractivity intercept	Difference	Specific dispersion	Refractivity intercept	Difference	Specific dispersion	Refractivity intercept	Difference	Specific dispersion	Refractivity intercept	Difference	Specific dispersion	Refractivity intercept	Difference
Benzene	2.05	1.78	0.27	0.54	0.40	0 14	1.94	1.90	0.04	0.24	0.16	0.08	0.16	0.15	0.01
Toluene	0.88	0.82	0.06	0.52	0.44	0.08	9.72	9.37	0.35	1.14	0.87	0.27	0.81	0.55	0.26
Ethylbenzene	0.16	0.12	0.04	0.32	0.23	0.09	0.37	0.34	0.03	0.38	0.25	0.13	0.31	0.20	0.11
m-Xylene } *p*-Xylene }	0.54	0.50	0.04	0.43	0.33	0.10	3.52	3.42	0.10	1.33	1.15	0.18	0.81	0.55	0.26
o-Xylene	0.24	0.19	0.05	0.15	0.12	0.03	1.55	1.50	0.05	0.64	0.54	0.10	0.24	0.13	0.11
Isopropylbenzene	0.08	0.04	0.04	0.09	0.06	0.03	..	..	..	0.19	0.12	0.07	0.22	0.10	0.12
n-Propylbenzene	0.22	0.21	0.01	0.21	0.18	0.03	..	..	..	0.52	0.45	0.07	0.41	0.34	0.07
	Jennings			KMA (Strawn)			M nument			Old Ocean			Plymouth		
Benzene	1.03	0.94	0.09	0.24	0.24	0.00	0.13	0.08	0.05	1.02	0.92	0.10	0.16	0.11	0.05
Toluene	3.51	3.45	0.06	1.04	0.90	0.14	0.48	0.30	0.18	2.69	2.49	0.20	0.72	0.62	0.10
Ethylbenzene	0.48	0.41	0.07	0.34	0.30	0.04	0.44	0.33	0.11	0.51	0.43	0.07	0.35	0.32	0.03
m-Xylene } *p*-Xylene }	3.48	3.38	0.10	1.19	1.05	0.14	0.50	0.31	0.19	2.79	2.46	0.33	0.75	0.58	0.17
o-Xylene	1.87	1.86	0.01	0.52	0.43	0.09	0.08	0.03	0.05	1.60	1.35	0.25	0.31	0.25	0.06
Isopropylbenzene	0.42	0.35	0.07	0.17	0.15	0.02	0.18	0.11	0.07	..	..	..	0.19	0.15	0.04
n-Propylbenzene	0.87	0.74	0.13	0.55	0.52	0.03	0.48	0.41	0.07	..	..	..	0.32	0.32	0.00
	Saxet			Segno			Slaughter			Wade City			Wasson		
Benzene	0.00	0.05	−0.05	0.59	0.54	0.05	4.60	4.12	0.48	0.10	0.08	0.02	2.29	1.98	0.31
Toluene	0.00	0.06	−0.06	3.22	3.07	0.15	4.80	4.50	0.30	0.79	0.66	0.13	4.44	4.16	0.28
Ethylbenzene	0.49	0.40	0.09	0.42	0.35	0.07	1.10	0.83	0.27	0.54	0.45	0.09	0.91	0.76	0.15
m-Xylene } *p*-Xylene }	0.58	0.44	0.14	3.07	2.86	0.21	2.86	2.78	0.08	1.43	1.24	0.19	3.13	2.91	0.22
o-Xylene	0.07	0.06	0.01	1.67	1.54	0.13	0.78	0.72	0.06	0.91	0.75	0.16	1.19	1.05	0.14
Isopropylbenzene	0.16	0.00	0.16	0.48	0.41	0.07	0.34	0.27	0.07	0.29	0.21	0.08	0.47	0.39	0.08
n-Propylbenzene	0.27	0.26	0.01	0.88	0.72	0.16	0.81	0.74	0.07	0.74	0.71	0.03	0.85	0.79	0.06

The diameter of the wire packing is 22 mm. The still pot is a 5-liter flask with a spherical ground-glass joint. The vacuum jacket requires no auxiliary heating when the column is operating below 320° F. A total condenser fits into the top of the column and provides reflux and liquid overhead product. The fractionating efficiency of this column at total reflux is 50 theoretical plates at the vaporization rate used in these distillations. In all columns the temperature of the vapor at the top of the column is measured by means of iron-constantan thermocouples with a precision of 0.3° F. (0.17° C.). The peaks for particular compounds in the plot of volume per cent against temperature as measured by the thermocouples usually agree with the literature values for boiling points of the particular compounds to 1° F.

Refractive index measurements are made in a Bausch & Lomb precision oil refractometer using monochromatic light. Light is provided by commercially available lamps at the wave lengths of the mercury *g* line (4358 Å.) and the sodium D line (5893 Å.). The precision of these measurements is ±0.00010 unit of refractive index for routine determinations. Specific gravities are determined at 20° C./20° C. with a chainomatic Westphal balance using a 2-ml. glass plummet. The precision of these measurements is ±0.0002 unit of specific gravity. Specific gravities are converted to densities at 20° C.

A measured volume of the sample is introduced into the still pot. The volume of this charge may be from 2 to 4 liters, but usually is between 3 and 3.5 liters. The column is preflooded to increase separating efficiency, and then allowed to operate at the still-pot pressure maintained throughout the distillation for 1 hour or longer. Product withdrawal is begun after this stabilizing period, and is adjusted to give a take-off rate of 1 ml. per minute. A reflux ratio of 20 to 1 is maintained by an appropriate vaporization rate throughout the distillation. Fractions are collected every 8 or 9 minutes, and the temperature of the vapor at the top of the column is recorded at this time. This top temperature is considered as the uncorrected boiling point of the last portion of the material in one fraction, and of the first portion of the succeeding fraction. Distillation is continued until the corrected boiling point exceeds 320° F., or until the still pot is empty. After the distillation is stopped, the still pot is cooled, the column is allowed to drain, the residue is removed and its volume measured. Each distillation yields from 200 to 400 fractions, each representing approximately 0.25% by volume of the original charge.

The volume, the specific gravity, and the refractive index for the mercury *g* line and the sodium D line are determined for each fraction.

DETERMINATION OF AROMATICS AND NAPHTHENES

Refractive indices and densities of mixtures of aromatics with naphthenes, with paraffins, or with mixtures of naphthenes and paraffins are not additive either on a volume per cent basis or a weight per cent basis, but their additivity is more nearly linear using volume per cent as shown in Table II. For mixtures of aromatics, naphthenes, and paraffins the specific dispersions likewise are not additive, but are more nearly so using a weight per cent basis as shown by Figure 1. For this reason the determination of aromatics by the use of specific dispersions is made on a weight per cent basis and this weight per cent is then converted to volume per cent.

The equation for weight per cent aromatics in a fraction is

$$W = \frac{S_f - S_{np}}{S_a - S_{np}} 100 + C \qquad (3)$$

where W is weight per cent aromatics, S_f is the specific dispersion of the fraction, S_a is specific dispersion of the individual aromatic

Table II. Nonadditivity of Density and Refractive Index for Mixtures of Toluene with *n*-Heptane and with Methylcyclohexane

Composition of Blends in Terms of Nonaromatic Component		Deviations of Experimental Densities and Refractive Indices from Theoretical (Theoretical-Experimental × 10^4)			
		Weight % Basis		Volume % Basis	
Weight % *n*-heptane	Volume %[a] *n*-heptane	d_4^{20}	n_D^{20}	d^{20}	n_D^{20}
100.00	100.00	0	0	0	0
98.98	99.19	5	3	2	1
96.98	97.60	13	8	2	1
94.97	95.98	20	13	2	2
89.97	91.89	38	25	3	5
79.98	83.50	69	45	5	7
70.17	74.85	92	61	7	10
50.30	56.15	113	76	7	13
29.98	35.13	100	69	6	13
19.99	24.02	77	54	4	10
10.02	12.35	43	32	1	7
5.02	6.27	23	17	1	4
3.03	3.80	13	11	−1	3
1.01	1.27	4	4	−1	1
0.00	0.00	0	0	0	0
Methylcyclohexane					
100.00	100.00	0	0	0	0
98.87	99.00	5	2	3	1
94.87	95.41	12	9	7	5
89.91	90.93	23	15	13	7
69.91	72.32	53	35	24	17
49.94	52.87	56	41	27	20
30.04	32.56	46	36	23	18
10.03	11.15	21	16	10	8
5.03	5.62	10	9	4	4
1.06	1.19	3	2	1	1
0.00	0.00	0	0	0	0

[a] Calculated from weight and density of components.

Table III. Boiling Points, Specific Dispersions, and Densities of Aromatics

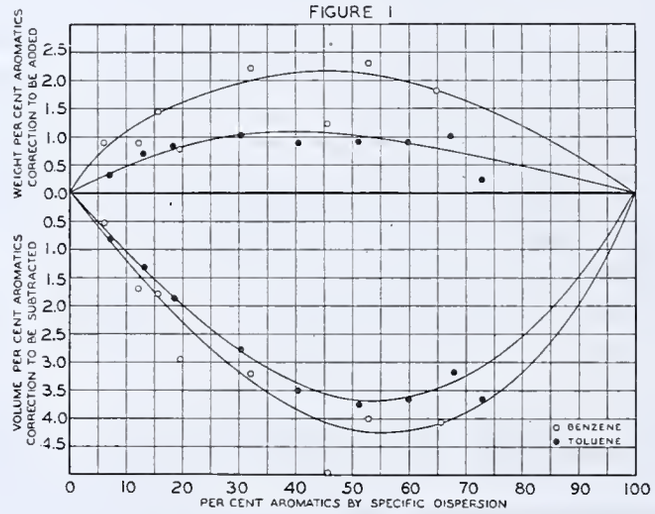
Aromatic	Boiling Point[a], 760 Mm. ° C.	° F.	Specific Dispersion[b], $(g - D)$	Density[b], 20° C.
Benzene	80.10	176.18	248.4	0.8790
Toluene	110.62	231.12	241.4	0.8669
Ethylbenzene	136.19	277.14	228.1	0.8671
p-Xylene	138.35	281.03	238.2	0.8610
m-Xylene	139.11	282.40	237.1	0.8641
o-Xylene	144.42	291.96	234.8	0.8800[a]
Isopropylbenzene	152.40	306.32	215.8	0.8619
n-Propylbenzene	159.22	318.60	216.0[c]	0.8619[a]

[a] From reliable but restrieted data.
[b] See (*8*).
[c] Estimated value.

being determined, S_{np} is the average value of the specific dispersions of the naphthenes and paraffins, and C is an additive correction for the deviation from linearity of the property-composition relationship. The value of S_f is calculated from the refractive indices and density of the fraction by the equation

$$S_f = \frac{n_g - n_D}{d_f} \times 10^4 \qquad (4)$$

where n_g and n_D are the refractive indices of the fraction for the mercury g line and the sodium D line, and d_f is the density of the fraction.



The value of S_{np} is 122.4, the values of S_a are listed in Table III, and the value of C is taken from the curves in Figure 2. A discussion of these values is included in the paper by Thorne, Murphy, and Ball (*8*). The equation for converting weight per cent aromatics to volume per cent is

$$V = \frac{(W)(d_f)}{d_a} \qquad (5)$$

where V is volume per cent aromatics, d_f is the density of the fraction, and d_a is the density of the aromatic compound, given in Table III. The calculations for the per cent aromatics in each fraction are recorded to the nearest figure in the first decimal place.

For the naphthene determination, refractivity intercepts for each fraction are calculated by the use of Equation 2 and are then plotted with their corresponding densities on the proper member of a series of 14 graphical analysis charts extending over the temperature range from 97° to 320° F. The corrected boiling point determines which of the charts will be used in plotting the property values of the fraction, and the volume per cent of naphthenes in the fraction is read directly from the plot of these values on the chart. Percentages for the naphthenes are recorded only in whole numbers.

APPLICATION OF THE METHODS OF ANALYSIS

The distillation characteristics of mixtures of aromatics with naphthenes and paraffins are such that most of the individual

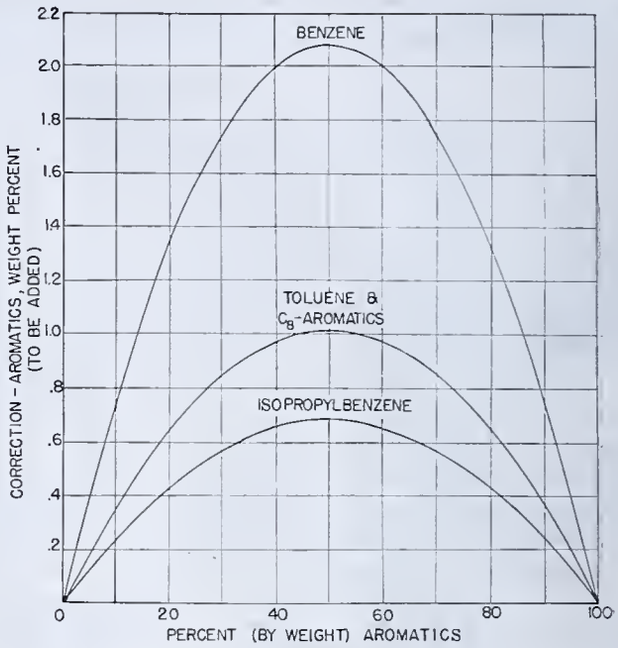


Figure 2. Curves for Correcting Aromatic Contents as Determined by Specific Dispersion Method

aromatic compound is distilled from a naphtha before the boiling point of the aromatic compound has been reached. Examination of the data from a large number of distillations has indicated the temperature limits within which the individual aromatic compounds are likely to be found. These limits for the individual compounds are: benzene, 146° to 185° F.; toluene, 203° to 251° F.; ethylbenzene, 251° to 273° F.; *p*-xylene and *m*-xylene, 273° to 283° F.; *o*-xylene, 283° to 295° F.; isopropylbenzene, 295° to 310° F.; and *n*-propylbenzene, 310° to 320° F. Equations 3, 4, and 5 are applied to all fractions within these ranges which have specific dispersions above 122.4, using the appropriate constants given in Table III for each aromatic compound. Because of the small difference in boiling point between *p*-xylene and *m*-xylene, making separation impossible under the distillation conditions employed, the constants for these two compounds are averaged, and the results of the determination for the range from 273° to 283° F. are expressed in terms of the mixture of the two. As indicated in Table III, the specific dispersion for *n*-propylbenzene was estimated, as none of the pure compound was available to make an experimental determination possible. However, since the densities and refractive indices (n_D) of isopropylbenzene and *n*-propylbenzene are practically the same, and since the specific dispersions for these two compounds calculated from the refractive indices for the hydrogen F and C lines are virtually identical (166.5 and 166.4), this estimated value of 216.0 should be within the experimental error of the true value, and the correction curve for isopropylbenzene in Figure 2 is used for *n*-propylbenzene. The summation of the percentages of aromatic in each fraction, based on the total amount of sample, yields the amounts of the individual aromatics in the sample.

To obtain greater precision in the determination of the naphthene content of each fraction, individual values for the refractivity intercepts of the pure compounds are used rather than the average values suggested by Kurtz and Headington.

Up to the present time the analysis of virgin naphthas has indicated that only those naphthenes that have 5- and 6-carbon-atom rings occur in these naphthas in detectable quantities. Such analyses have also indicated the absence of the *cis* form of the naphthene compounds, and the absence of the more highly branched paraffin hydrocarbons. This means that only about 34 individual aromatic and saturated hydrocarbons need be considered in the analysis of a naphtha in that portion of the material boiling between 97° and 243° F. From the above considerations and from information in the literature, about 70 compounds

should be considered in that portion of the naphtha distilling between 243° and 320° F.

A study of the boiling points of the compounds in the 97° to 320° F. range indicates certain fairly wide temperature intervals at which no material should distill, so that an efficient fractional distillation should show minima in the amount of distillate recovered at these temperature intervals. Examination of the data from more than 75 such distillations shows that such minima do occur, suggesting the use of these points as temperature divisions in the method of analysis.

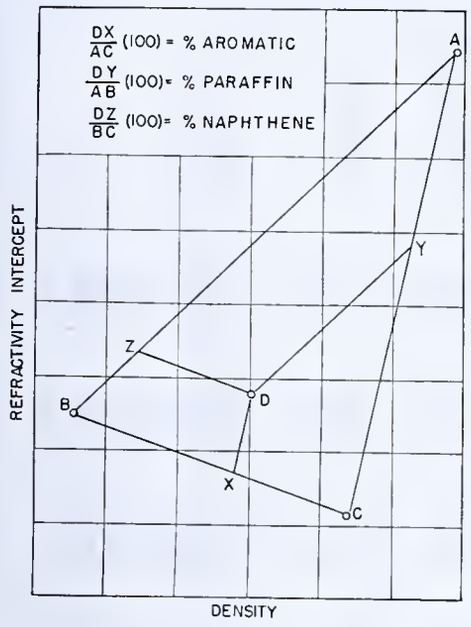


Figure 3. Graphical Analysis Diagram

On the basis of these points of minima and from a knowledge of the refractivity intercepts and densities of the individual hydrocarbons, together with a consideration of the boiling points and distillation characteristics of the aromatic compounds, the temperature range from 97° to 320° F. has been divided into 15 smaller ranges for analytical purposes. Graphical analysis charts, using refractivity intercepts and densities, have been constructed for 14 of these temperature ranges. No chart is necessary for naphthene analysis in the range from 135° to 146° F., since only paraffin hydrocarbons are present. Table IV indicates the temperature range of each division and the individual compounds, together with their physical properties, which have been used in defining the 14 charts.

If points A, B, and C in Figure 3 represent the refractivity intercept and density of a pure individual aromatic, paraffin, and naphthene hydrocarbon, respectively, then the composition of any fraction, D, containing only these 3 hydrocarbons can be determined from the geometrical position of point D within the triangle formed by points A, B, and C, provided that the properties which define each of the points are additive with relation to composition. As there are only 8 aromatic compounds in the boiling range considered, it was relatively easy to locate the aromatic points (A in Figure 3) on those of the 14 charts where aromatics must be considered.

For a number of boiling ranges the paraffins and naphthenes probably present are sufficiently well known and their constants sufficiently well established to make it possible to use their refractivity intercept and density values to establish points B and C in Figure 3, and therefore the slope and position of the paraffin-naphthene connecting line. Where there is insufficient information to permit this procedure, the slope and position of the line have been established by using data for distilled fractions free from aromatic material. The length of the line, and therefore the location of points B and C, are determined by a consideration of the paraffins and naphthenes which theoretically may be present and those which the data indicate most probably are present or absent. Figures 4, 5, 7, 8, 9, 10, 12, 13, 14, 16, 17, and 19 show some of the representative data used in defining the paraffin-naphthene base line of the graphical analysis triangles. These data are not continuous from one figure to the next, except as indicated, and were taken from a large number of distillations.

Figures 6, 11, 15, and 18 represent the finished form of 4 of the 14 graphical analysis charts used for the naphthene determinations except for the omission here of the smaller scale divisions ordinarily used, and show data for 4 different naphthas. These naphthas are from Saxet, Yates and Taylor-Link, and Conroe crude oils from Texas, and from Lance Creek crude oil from Wyoming. Each is distinctive in its composition characteristics. The Saxet naphtha, from the Corpus Christi area of Texas, contains an unusually high percentage of the naphthenic hydrocarbons. The Yates and Taylor-Link mixed sample from West Texas, containing about 75% of Yates material, has an abnormally low percentage of normal paraffins and a high percentage of isomeric paraffins. The Conroe, Texas, naphtha has an exceptionally high content of the aromatic hydrocarbons. The Lance Creek, Wyo., naphtha was completely dearomatized by filtration through silica gel before the distillation was made. The 14 graphical analysis charts are scaled in 1% divisions for aromatics and in 2% increments for naphthenes. Each of the 15 temperature ranges from 97° to 320° F. is discussed in detail below.

Figure 4. Temperature range, 97° to 135° F. Cyclopentane is the only naphthene found in this range; n-pentane, 2,2-dimethylbutane, and 2,3-dimethylbutane are the paraffins present; and there is no aromatic. Cyclopentane, which occurs in most crude oils, usually is associated with a smaller quantity of 2,2-dimethylbutane. The total quantity of both compounds is always small. The temperature 135° F. seems to be the most suitable for the inclusion of all the cyclopentane in this range, and the exclusion of the major portion of the 2,3-dimethylbutane. In Figure 4 the paraffin and naphthene points, and therefore the connecting line, are defined by the appropriate constants for 2,2-dimethylbutane and cyclopentane. Two sets of data points for typical naphthas are also given by curves A and B, showing the change in composition of the successive fractions with increase in boiling point

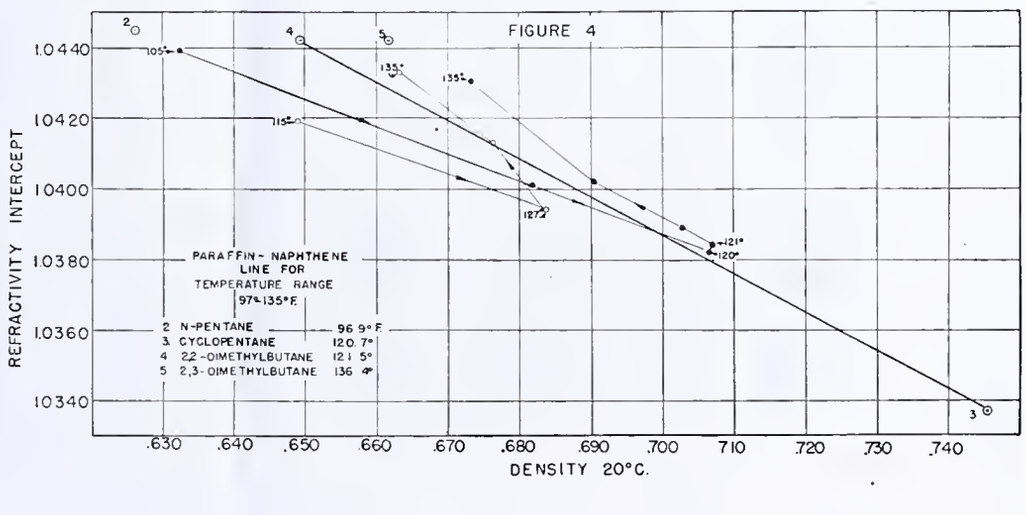
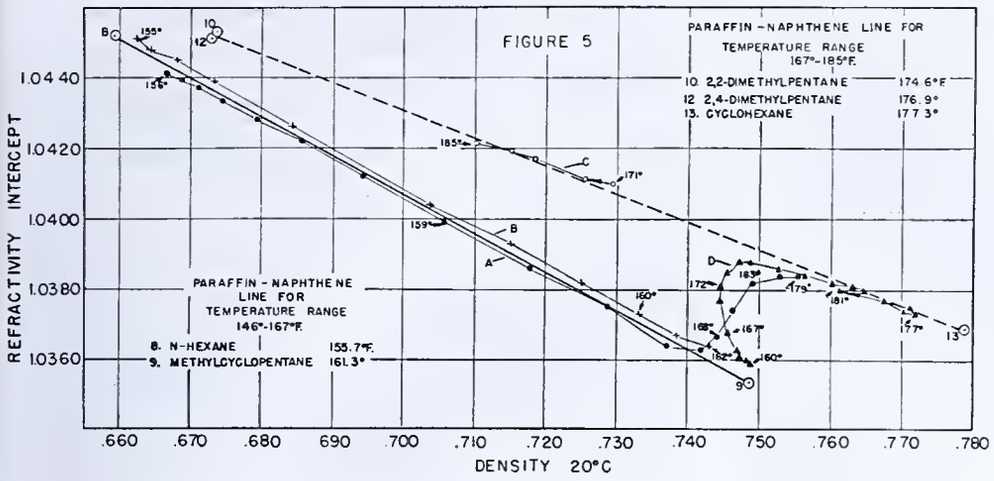
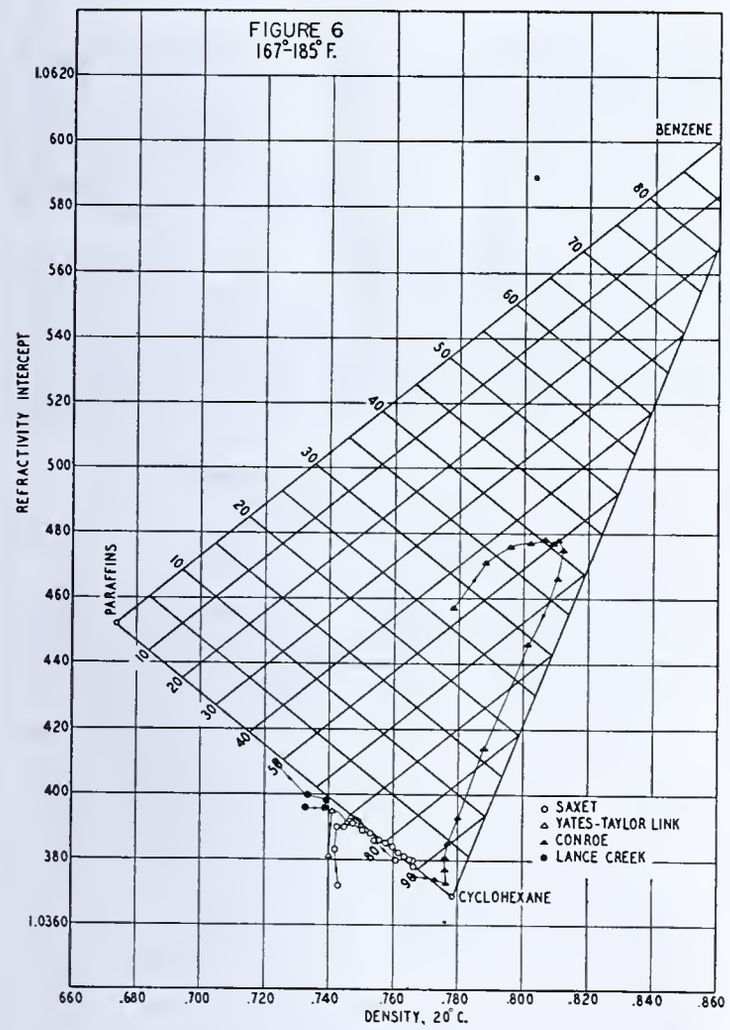


Table IV. Data Used in Constructing Refractivity Intercept–Density Charts

Chart No.	Range, ° F.	Aromatics: Hydrocarbon	Aromatics: Boiling point, ° F.	Aromatics: Density, 20° C.	Aromatics: Refractivity intercept	Naphthenes: Hydrocarbon	Naphthenes: Boiling point, ° F.	Naphthenes: Density, 20° C.	Naphthenes: Refractivity intercept	Paraffins: Hydrocarbon	Paraffins: Boiling point, ° F.	Paraffins: Density, 20° C.	Paraffins: Refractivity intercept
1	97–135	None	...	...	...	Cyclopentane	120.7	0.7454	1.0337	2,2-Dimethylbutane	121.5	0.6492	1.0442
	135–146	(All paraffins—no aromatics or naphthenes)											
2	146–167	Benzene	176.2	0.8790	1.0616	Methylcyclopentane	161.3	0.7486	1.0354	*n*-Hexane	155.7	0.6594	1.0452
3	167–185	Benzene	176.2	0.8790	1.0616	Cyclohexane	177.3	0.7786	1.0369	2,2-Dimethylpentane	174.6	0.6739	1.0453
										2,4-Dimethylpentane	176.9	0.6730	1.0451
			Av.	0.8790	1.0616			0.7786	1.0369			0.6735	1.0452
4	185–203	None	...	...	...	*trans*-1,3-Dimethylcyclopentane	195.4	0.745	1.0367	2,3-Dimethylpentane	193.6	0.6951	1.0445
						trans-1,2-Dimethylcyclopentane	197.4	0.752	1.0360	2-Methylhexane	194.1	0.6788	1.0455
										3-Methylhexane	197.5	0.6870	1.0452
							Av.	0.749	1.0364			0.6870	1.0451
5	203–215	Toluene	231.1	0.8668	1.0634	Methylcyclohexane	213.7	0.7694	1.0384	*n*-Heptane	209.2	0.6837	1.0459
6	215–222	Toluene	231.1	0.8668	1.0634	Ethylcyclopentane	218.2	0.7665	1.0366	2,2-Dimethylhexane	224.3	0.6953	1.0459
7	222–235	Toluene	231.1	0.8668	1.0634	1,2,4-Trimethylcyclopentane	235.0	0.7565	1.0373	2,2-Dimethylhexane	224.3	0.6953	1.0459
										2,4-Dimethylhexane	229.0	0.7003	1.0452
										2,5-Dimethylhexane	228.4	0.6935	1.0458
			Av.	0.8668	1.0634			0.7565	1.0373			0.6964	1.0456
8	235–243	Toluene	231.1	0.8668	1.0634	1,2,4-Trimethylcyclopentane	235.0	0.7565	1.0373	2,3-Dimethylhexane	240.1	0.7133	1.0449
9	243–251	Toluene	231.1	0.8668	1.0634	*trans*-1,3-Dimethylcyclohexane	248.2	0.7660	1.0399	2-Methylheptane	243.8	0.6979	1.0461
						trans-1,4-Dimethylcyclohexane	246.8	0.7625	1.0397	3-Methylheptane	246.1	0.7058	1.0456
						trans-1-Methyl-2-ethylcyclopentane	250.7	0.7696	1.0372	4-Methylheptane	243.9	0.7046	1.0456
			Av.	0.8668	1.0634			0.7660	1.0389			0.7028	1.0458
10	251–273	Ethylbenzene	277.2	0.8669	1.0624	*trans*-1,2-Dimethylcyclohexane	254.2	0.7760	1.0390	*n*-Octane	258.2	0.7026	1.0462
						Propylcyclopentane	267.4	0.7761	1.0385				
						Isopropylcyclopentane	259.5	0.7763	1.0378				
						Ethylcyclohexane	269.2	0.7879	1.0391				
			Av.	0.8669	1.0624			0.7791	1.0386			0.7026	1.0462
11	273–283	*p*-Xylene	281.0	0.8610	1.0653	1,1,3-Trimethylcyclohexane	277.4	0.7792	1.0398	2,5-Dimethylheptane	276.9	0.715	1.0462
		m-Xylene	282.4	0.8641	1.0652	1-Methyl-3-isopropylcyclopentane	279.9	0.7771	1.0392	2,6-Dimethylheptane	275.4	0.7089	1.0463
						1,3-Dimethyl-2-ethylcyclopentane	280.4	0.7756	1.0396	3,3-Dimethylheptane	279.2	0.725	1.0460
			Av.	0.8626	1.0653			0.7773	1.0395			0.7163	1.0462
12	283–295	*o*-Xylene	292.0	0.8800	1.0653	1-Methyl-2-isopropylcyclopentane	288.5	0.7792	1.0383	2-Methyloctane	289.9	0.7134	1.0464
						tert-Butylcyclopentane	293.4	0.7911	1.0385	3-Methyloctane	291.5	0.7207	1.0459
						1,2,4-Trimethylcyclohexane	286.2	0.7720	1.0406	4-Methyloctane	288.5	0.7199	1.0462
						trans-1,2,3-Trimethylcyclohexane	288.5	0.7914	1.0400				
			Av.	0.8800	1.0653			0.7834	1.0393			0.7180	1.0462
13	295–310	Isopropylbenzene	306.3	0.8617	1.0605	*trans*-1,2-Diethylcyclopentane	297.5	0.7832	1.0379	*n*-Nonane	303.4	0.7178	1.0465
						Isobutylcyclopentane	298.9	0.7807	1.0396				
						trans-1-Methyl-3-ethylcyclohexane	299.3	0.790	1.0394				
						1,3-Diethylcyclopentane	299.3	0.7850	1.0373				
						1-Methyl-4-ethylcyclohexane	302.2	0.790	1.0393				
						1,1-Diethylcyclopentane	302.9	0.8027	1.0374				
						1-Methyl-2-ethylcyclohexane	306.7	0.804	1.0368				
						Isopropylcyclohexane	309.9	0.8016	1.0400				
						sec-Butylcyclopentane	310.3	0.7941	1.0390				
			Av.	0.8617	1.0605			0.7924	1.0385			0.7178	1.0465
14	310–320	*n*-Propylbenzene	318.6	0.8619	1.0610	Propylcyclohexane	314.1	0.7933	1.0403	2,6-Dimethyloctane	317.5	0.7291	1.0461
						Butylcyclopentane	315.0	0.7847	1.0386	3,5-Dimethyloctane	317.9	0.7328	1.0467
										2,5-Dimethyloctane	318.2	0.7349	1.0453
										2,7-Dimethyloctane	320.0	0.7247	1.0468
			Av.	0.8619	1.0610			0.7890	1.0394			0.7304	1.0462





few fractions, but will have a negligible effect on the naphthene determination. The temperature 167° F. is a point showing a minimum in the amount of distillate recovered in the distillations, and also serves as a division between methylcyclopentane in this temperature range and a possible azeotrope formed by benzene and cyclohexane in the following range. This mixture has a boiling point of about 170° F. Curves *A* and *B* in Figure 5 indicate clearly the slope and position of the base line for this temperature range and confirm the selection of the constants for *n*-hexane and methylcyclopentane as the paraffin and naphthene points. The data for this range indicate the high concentrations of the 2 hydrocarbons that can be expected in these distillations. The paraffin-naphthene base line shown in Figure 5 is combined with the aromatic point for benzene to obtain a triangle similar in form to the triangles shown in Figures 3 and 6.

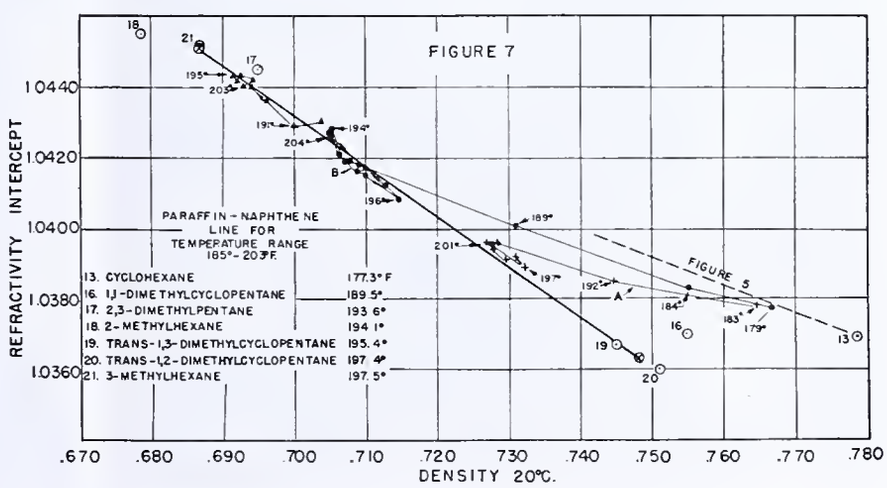
Figures 5 and 6. Temperature range, 167° to 185° F. Cyclohexane is the only naphthene boiling in this range and usually occurs with 2,2-dimethylpentane, 2,4-dimethylpentane, and benzene. A minimum in volume per cent recovered occurs at 185° F. This is also the point at which virtually all of the cyclohexane has distilled from the sample. The bases of two temperature ranges are shown together in Figure 5 to indicate the shift in naphthenic constituents in the course of the distillation from methylcyclopentane to cyclohexane and also to show the probable presence of either or both 2,2-dimethylpentane and 2,4-dimethylpentane. Curve *D* in Figure 5 shows that in some naphthas the paraffin concentration of the fractions increases markedly between the maximum concentrations of these two naphthenes. The boiling points of these fractions and of the pure compounds indicate that this increase must be caused by either or both of the 2 dimethylpentanes above. Curve *C* shows an unusual 50% by volume concentration of paraffins in this range. The distillation data in Figures 5 and 6 indicate that the paraffin and naphthene points were chosen properly.

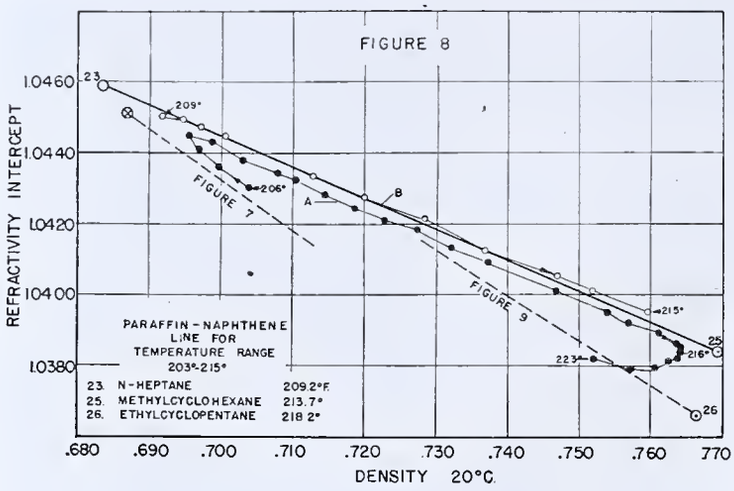
Figure 7. Temperature range 185° to 203° F. Naphthenes boiling in this range are 1,1-dimethylcyclopentane, *trans*-1,3-dimethylcyclopentane, and *trans*-1,2-dimethylcyclopentane, and the paraffins are 2,3-dimethylpentane, 2-methylhexane, and 3-methylhexane. Present experience does not indicate the presence of either 3,3-dimethylpentane or 3-ethylpentane. The amounts of benzene or toluene found are negligible and their presence is unusual. The temperature 203° F. is another point in the distillations where a definite minimum in total volume per cent occurs, and is also a division between the isomeric heptanes and *n*-heptane. It is the lowest temperature at which toluene first appears in any of the distillations. Curves *A* and *B* in Figure 7 show the shift with increase in boiling point from cyclohexane in Figure 5 toward 2,3-dimethylpentane and 2-methylhexane in Figure 7, a reversal toward the 2 higher-boiling *trans*-dimethylcyclopentanes, and a final reversal in the paraffin direction toward 3-methylhexane. The required slope and position of the paraffin-naphthene line is defined by the data in Figure 7 and the paraffin and naphthene points selected fulfill these requirements. The paraffin point is an average of the values for 2,3-dimethylpentane, 2-

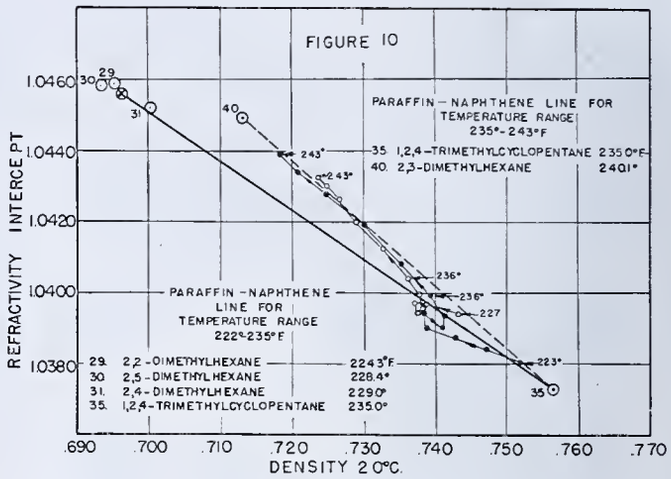
from *n*-pentane through mixtures containing cyclopentane to 2,3-dimethylbutane. Since no aromatic occurs in this range the paraffin-naphthene line suffices for the naphthene determination.

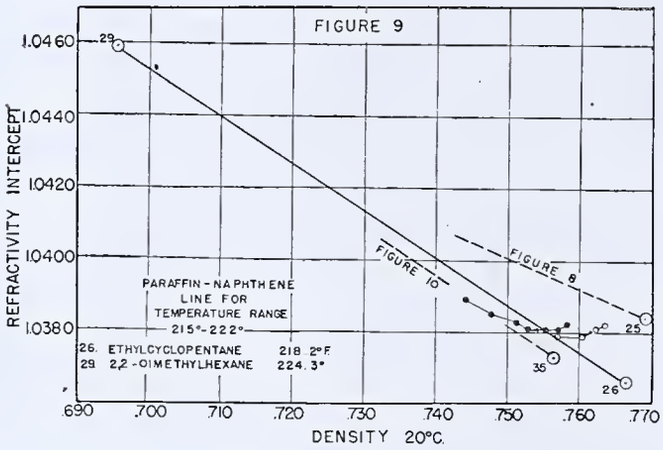
Temperature range, 135° to 146° F. Normally all material boiling in this range will be paraffinic, and no naphthene determination is required. The paraffins present are 2,3-dimethylbutane, 2-methylpentane, and 3-methylpentane and a procedure for estimating their relative quantities, based on refractive indices and densities, is outlined later. The temperature 146° F. was selected as the maximum that can be reached in all distillations without including some benzene.

Figure 5. Temperature range, 146° to 167° F. Methylcyclopentane, *n*-hexane, and benzene are the compounds usually found in this range. Some 3-methylpentane may occur in the first









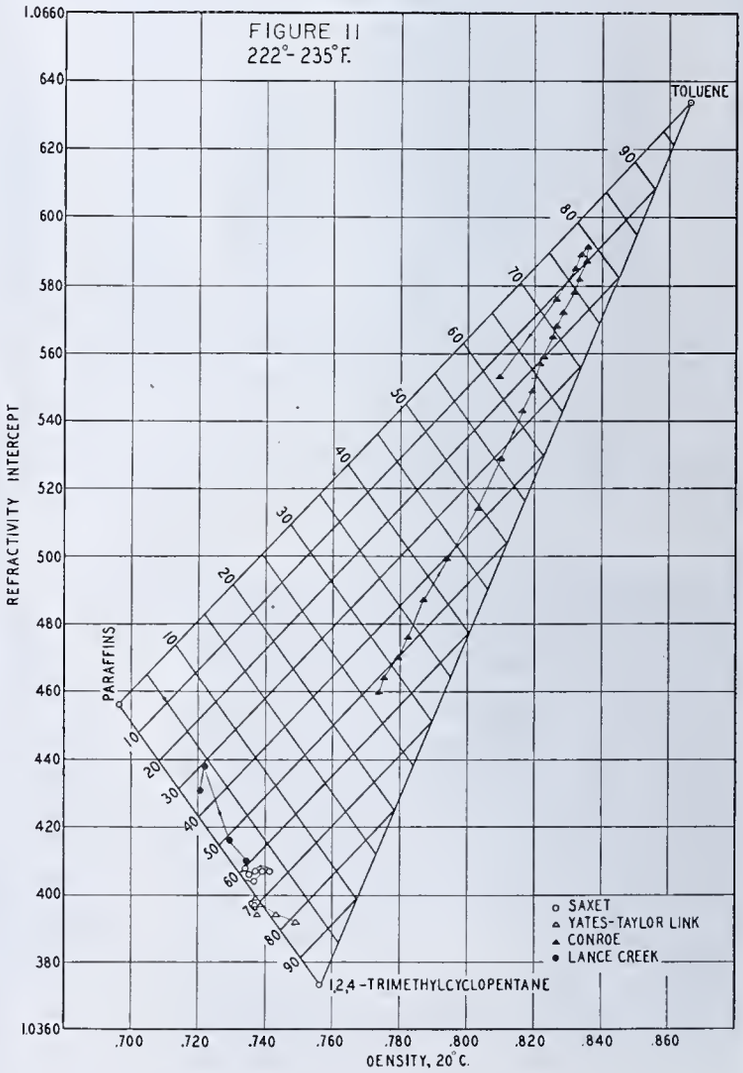
methylhexane, and 3-methylhexane and the naphthene point is an average of the values for *trans*-1,3-dimethylcyclopentane and *trans*-1,2-dimethylcyclopentane. The quantity of 1,1-dimethylcyclopentane in this range relative to the total amount of the other 2 naphthenes is usually small and consequently its constants were not used in the average for the naphthene point.

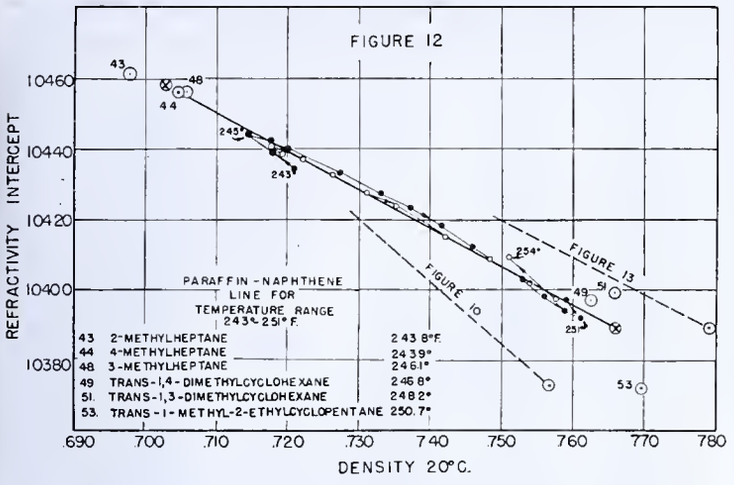
Figure 8. Temperature range, 203° to 215° F. Methylcyclohexane, *n*-heptane, and toluene are the only hydrocarbons usually present in this range. The temperature 215° F. separates methylcyclohexane from ethylcyclopentane in Figure 9, and *n*-heptane from the isomeric octanes. Curve *A* in Figure 8 shows the transition from Figure 7 to *n*-heptane in Figure 8. All data for this range show a change in concentration from *n*-heptane toward methylcyclohexane, both hydrocarbons usually occurring in high concentrations. Curve *A* in Figure 8 also shows a shift from methylcyclohexane in Figure 8 toward Figure 9. All the data indicate that the constants for methylcyclohexane and *n*-heptane define the paraffin-naphthene line for this range very accurately.

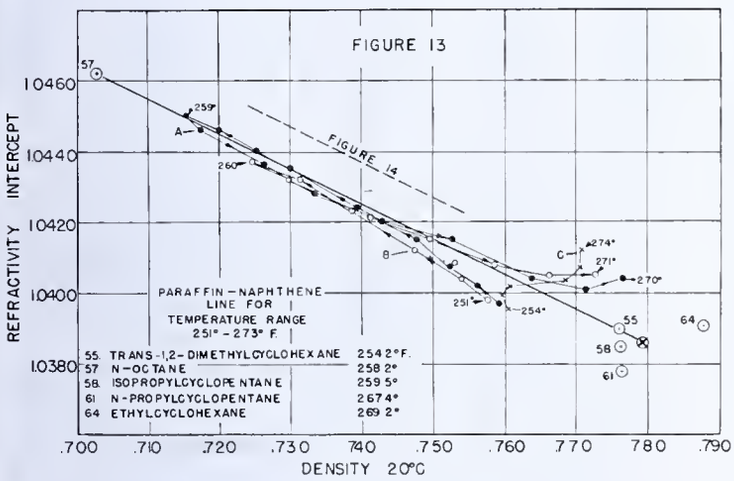
Figure 9. Temperature range, 215° to 222° F. Ethylcyclopentane, 2,2-dimethylhexane, and toluene are the hydrocarbons indicated in this range. The temperature 222° F. is at a point showing a minimum in volume per cent of distillate and separates ethylcyclopentane from the higher-boiling naphthenes in Figure 10. The data shown in Figure 9 indicate that only a few cuts are normally recovered in this range and that these cuts contain a high naphthene concentration. The napthene point for the paraffin-naphthene line is based on the properties of ethylcyclopentane and the paraffin point is based on the properties of 2,2-dimethylhexane, although its boiling point is higher than the indicated temperature range of Figure 9.

Figures 10 and 11. Temperature range, 222° to 235° F. Two or more unidentified naphthenes probably are present in this range together with the paraffins and toluene. The probable paraffins are 2,2-dimethylhexane, 2,5-dimethylhexane, and 2,4-dimethylhexane, with no indication of the presence of 3,3-dimethylhexane. The temperature 235° F. usually indicates a change in the paraffin constituents of the fractions boiling at this point, making a change in the paraffin point of the paraffin-naphthene line advisable. The data for this range in Figures 10 and 11 indicate a small amount of distillate throughout the range except in those cases where the amount of toluene in the sample is high. Since the amount of distillate in the range is usually small for naphthas having no toluene in them, the paraffin-naphthene line cannot be defined as accurately as in some of the other ranges. Figure 11 represents the best solution possible with present knowledge and data. The naphthene point is based on the constants for 1,2,4-trimethylcyclopentane, although its actual presence is uncertain. The paraffin point is determined by the average of the values for 2,2-dimethylhexane, 2,5-dimethylhexane, and 2,4-dimethylhexane.

Figure 10. Temperature range, 235° to 243° F. The naphthene material in this range is characterized by the properties of 1,2,4-trimethylcyclopentane and the probable paraffin is 2,3-dimethylhexane. Toluene may be present in small quantities. Naphthene and paraffin concentration relative to the whole sample normally begins to increase rapidly at 243° F. and a change in both naphthene and paraffin points is indicated by the distillation data.





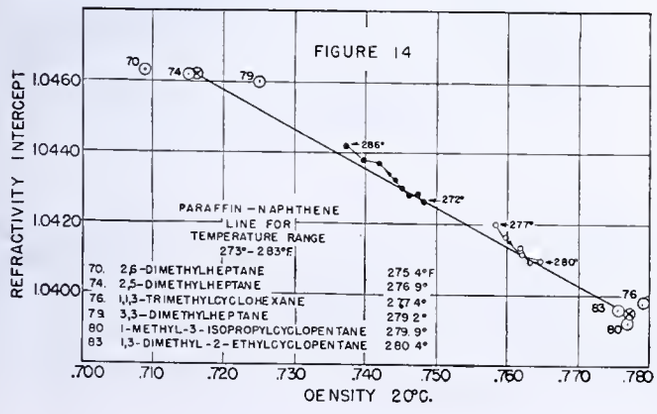


The slope and position of the paraffin-naphthene line are well defined and there are no reversals of direction in the data. The naphthene point is determined by the constants for 1,2,4-trimethylcyclopentane and the paraffin point by the constants for 2,3-dimethylhexane. Here again, the occurrence of 1,2,4-trimethylcyclopentane is uncertain, and more than one naphthene actually may be present.

Figure 12. Temperature range, 243° to 251° F. The predominant naphthenes in this region are *trans*-1,3-dimethylcyclohexane and *trans*-1,4-dimethylcyclohexane, with possibly some 1,1-dimethylcyclohexane, and one or more cyclopentane derivatives with constants similar to those of *trans*-1-methyl-2-ethylcyclopentane. The predominant paraffins are 2-methylheptane, 4-methylheptane, and 3-methylheptane. Small amounts of toluene may be present in some distillations. The temperature 251° F. serves to separate the isomeric octanes from *n*-octane, and the naphthenes in this region from *trans*-1,2-dimethylcyclohexane in Figure 13. The slope and position of the paraffin-naphthene line are well defined by the data in Figure 12. The naphthene point is based on the average of the refractivity intercepts and densities of *trans*-1,4-dimethylcyclohexane, *trans*-1,3-dimethylcyclohexane, and *trans*-1-methyl-2-ethylcyclopentane. Probably some 1,1-dimethylcyclohexane is present but the relative amount is usually small. The paraffin point is defined by the average of the values for 2-methylheptane, 4-methylheptane, and 3-methylheptane. The data for this range confirm this selection of paraffin and naphthene points.

Figure 13. Temperature range, 251° to 273° F. The principal naphthenic constituents in this range appear to be *trans*-1,2-dimethylcyclohexane, *n*-propylcyclopentane, isopropylcyclopentane, and ethylcyclohexane. The paraffin usually present in greatest concentration is *n*-octane, although small amounts of 2,-2-dimethylheptane and 2,4-dimethylheptane may occur. Ethylbenzene is usually present in this range. The temperature 273° F. serves as a division between ethylcyclohexane in this range and the naphthenes in Figure 14, and also as a relative division between ethylbenzene and the 2 aromatics in the next boiling range. Curves *A* and *B* in Figure 13 show a high concentration of naphthenic material in the first fractions of the range, the trend of the data toward the paraffin point because of the presence of *n*-octane, and the reversal near the boiling point of *n*-octane toward another region of high naphthenic concentration. Curves *A*, *B*, and *C* show the shift from Figure 13 toward Figure 14 in the higher-boiling fractions in this range. Curve *C* represents a naphtha almost devoid of the normal paraffins. The data define the paraffin-naphthene line rather well and indicate that the paraffin and naphthene points are properly located. The naphthene point is defined by an average of the constants for *trans*-1,2-dimethylcyclohexane, isopropylcyclopentane, *n*-propylcyclopentane, and ethylcyclohexane. The paraffin point is represented by the values for *n*-octane.

Figures 14 and 15. Temperature range, 273° to 283° F. There is no definite indication of the probable naphthenes in this range and the more probable paraffins are 2,6-dimethylheptane, 2,5-dimethylheptane, and 3,3-dimethylheptane. *p*-Xylene and *m*-xylene usually are present in the fractions distilling within the above temperatures. The temperature 283° F. is used to separate the dimethylheptanes from the methyloctanes, and *p*-xylene and *m*-xylene from *o*-xylene in the following temperature range. One set of data in Figure 14 shows an increase in paraffin concen-



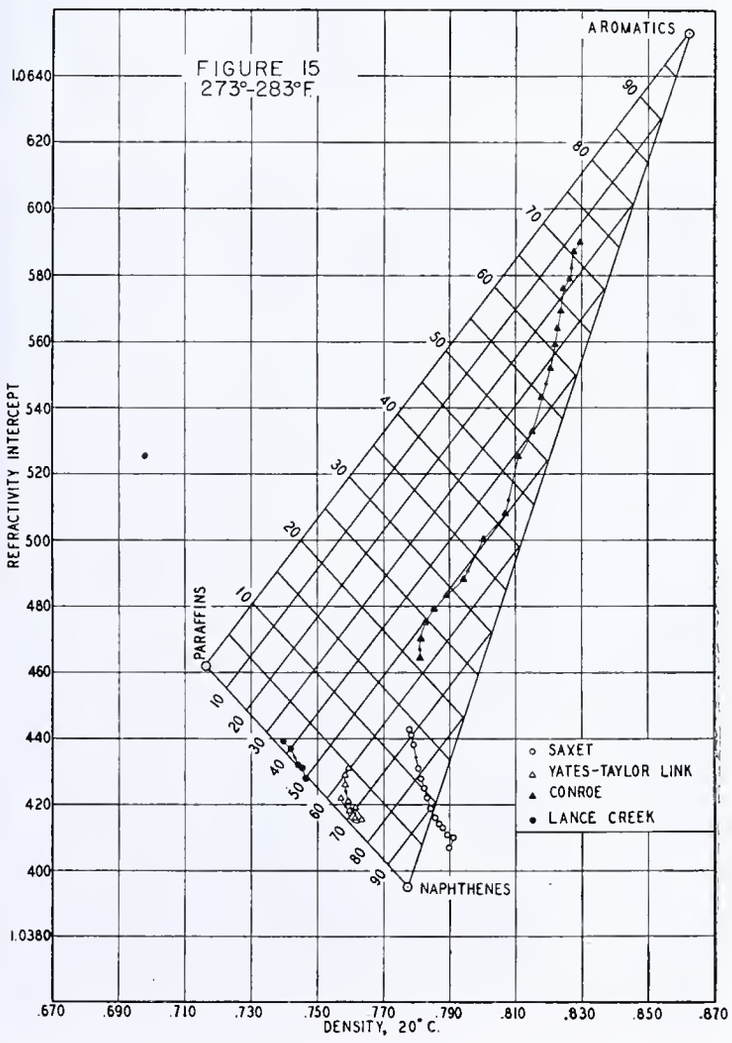
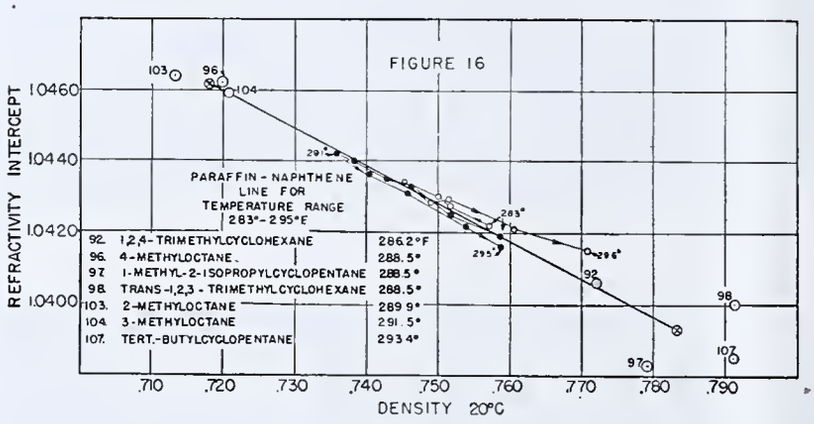


Table V. Volume Per Cent of Individual Hydrocarbons in the 97° to 243° F. Boiling Range for Several Representative Naphthas

Hydrocarbons	Coalinga	Conroe	Hastings	Jennings	KMA Strawn	Old Ocean	Saxet	Segno	Tom O'Connor	Wade City
n-Pentane[a]	0.44	0.33	0.89	1.12	0.40	0.36	1.11	0.55	None	1.28
Cyclopentane	1.76	0.96	1.23	0.67	1.25	0.85	0.73	1.08	1.04	1.36
2,2-Dimethylbutane	0.25	0.39	0.78	0.42	0.16	0.58	0.32	0.74	0.60	0.57
2,3-Dimethylbutane	2.21	1.46	1.97	2.05	1.74	1.88	1.25	1.94	2.09	1.27
2-Methylpentane	2.56	2.89	2.16	3.47	4.39	5.23	1.33	3.71	4.61	1.14
3-Methylpentane	1.89	2.09	1.81	1.03	3.80	3.36	0.88	2.22	2.62	1.52
n-Hexane	7.75	6.44	5.18	9.15	12.71	10.18	2.29	8.84	7.76	2.04
Methylcyclopentane	10.29	6.51	7.42	5.01	7.52	5.42	5.52	6.40	6.70	7.39
2,2-Dimethylpentane } 2,4-Dimethylpentane }	0.51	0.96	2.55	1.46	0.79	1.52	3.51	1.38	1.72	2.27
Benzene	2.22	3.27	0.16	3.61	0.59	2.28	None	1.82	0.58	0.38
Cyclohexane	7.63	10.40	13.66	7.13	4.68	7.30	15.07	9.64	10.94	14.93
1,1-Dimethylcyclopentane	1.17	0.35	0.70	1.00	0.45	0.83	1.30	0.59	0.68	0.96
2,3-Dimethylpentane } 2-Methylhexane }	2.69	3.45	4.53	6.14	5.81	6.71	2.39	5.32	5.79	2.27
trans-1,3-Dimethylcyclopentane	4.92	2.62	5.78	2.53	7.08	3.87	4.33	2.98	4.36	3.97
trans-1,2-Dimethylcyclopentane	7.05	1.59	2.35	1.02	5.29	1.41	3.12	1.29	2.64	3.94
3-Methylhexane	3.30	1.90	1.49	2.25	3.90	2.88	1.25	2.01	3.29	1.32
n-Heptane	5.94	6.90	2.43	8.42	11.32	10.92	1.69	7.96	4.96	0.86
Methylcyclohexane	14.55	22.00	32.39	18.07	12.29	17.20	37.48	21.58	23.02	35.62
Ethylcyclopentane	4.38	2.03	3.58	2.34	3.65	3.48	4.09	2.70	3.22	5.16
2,2-Dimethylhexane	0.57	0.71	0.77	2.39	0.93	1.51	1.35	1.27	1.51	1.39
2,5-Dimethylhexane } 2,4-Dimethylhexane }	0.56	2.70	2.02	2.13	0.51	1.04	2.11	1.69	0.33	1.19
Toluene	7.94	16.19	0.77	12.02	2.57	5.87	None	9.61	3.39	2.64
Trimethylcyclopentanes (?)	7.23	3.56	3.05	3.71	4.56	3.38	4.42	2.56	3.58	3.69
2,3-Dimethylhexane	1.30	0.22	1.33	2.39	2.49	1.45	1.66	1.87	3.39	1.23
A trimethylcyclopentane (?)	0.89	0.08	1.00	0.47	1.12	0.49	2.80	0.25	1.18	1.61
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[a] *n*-Pentane boiling below 97.0° F. not included in these figures.

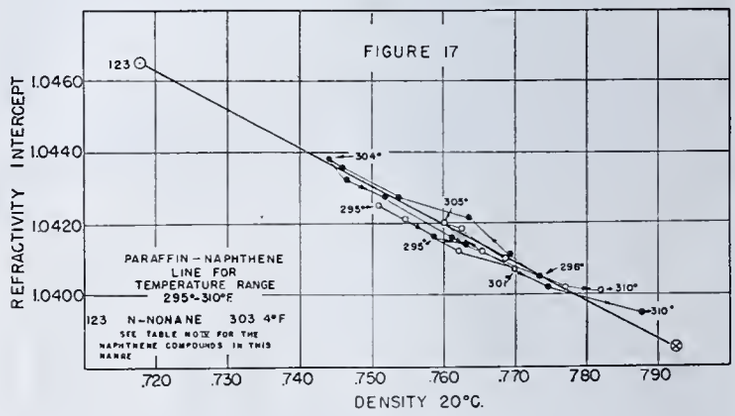


tration with an increase in boiling point whereas the other set shows the reverse. The paraffin-naphthene line seems to be reasonably correct although more knowledge of the constituents of this range is desirable. The naphthene point is defined by the refractivity intercepts and densities of the 3 naphthenes listed in Table IV for this range. These may or may not be the more probable hydrocarbons present. The paraffin point is defined by the average of the values for 2,6-dimethylheptane, 2,5-dimethylheptane, and 3,3-dimethylheptane. The aromatic point in Figure 15 is based on the average of the constants for *p*-xylene and *m*-xylene. For the C_8 and the two C_9 aromatics determined in this scheme of analysis, the temperature limits chosen for the individual compounds do not imply clear-cut separations of the hydrocarbons by the distillations, and the method of analysis relies on the principle of compensating errors for the attainment of the probable error later indicated for these determinations. The principle of compensating errors is included also in the accuracy indicated for the paraffin and naphthene determinations.

Figure 16. Temperature range, 283° to 295° F. The naphthenes in this range, as in the preceding range and the 2 succeeding ranges, are not well known and no attempt is made to single out the predominant naphthenic compounds. The paraffins are probably 4-methyloctane, 2-methyloctane, and 3-methyloctane. *o*-Xylene is usually present in this temperature range. The temperature 295° F. separates *o*-xylene from isopropylbenzene (cumene) and the isomeric nonanes from *n*-nonane. The slope and position of the paraffin-naphthene line are clearly indicated by the data in Figure 16. This confirms the location of the paraffin point and indicates that the naphthene point has been selected with reasonable accuracy. The paraffin point is based on the average of the constants for 4-methyloctane, 2-methyloctane, and 3-methyloctane. The naphthene point is defined by the average of the values for the 4 naphthenes listed in Table IV for this range.

Figures 17 and 18. Temperature range, 295° to 310° F. Some of the naphthenes that are theoretically constituents of the material boiling in this range are listed in Table IV. The predominant paraffin is *n*-nonane, and isopropylbenzene is usually present in this temperature range. The temperature 310° F. separates isopropylbenzene from *n*-propylbenzene and *n*-nonane from the isomeric decanes likely to be present in petroleum naphthas. The data in Figure 17 define the slope and position of the paraffin-naphthene line and indicate that the paraffin and naphthene points represent the largest part of previous distillation data. The paraffin point is the plot of the refractivity intercept and density of *n*-nonane, and the naphthene point is based on the average of the constants for the compounds listed in Table IV for this range.

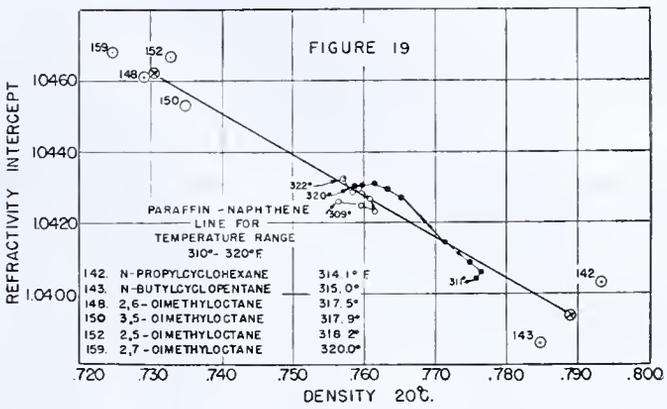
Figure 19. Temperature range, 310° to 320° F. The naphthenes in this range are represented by the values for *n*-propylcyclohexane and *n*-butylcyclopentane. The paraffins present are probably 2,6-dimethyloctane, 3,5-dimethyloctane, 2,5-dimethyloctane, and 2,7-dimethyloctane. *n*-Propylbenzene usually is present in the range. The temperature 320° F. was chosen to include all the *n*-propylbenzene in the naphthas. The data in Figure 19 do not define the paraffin-naphthene line as well as most of the



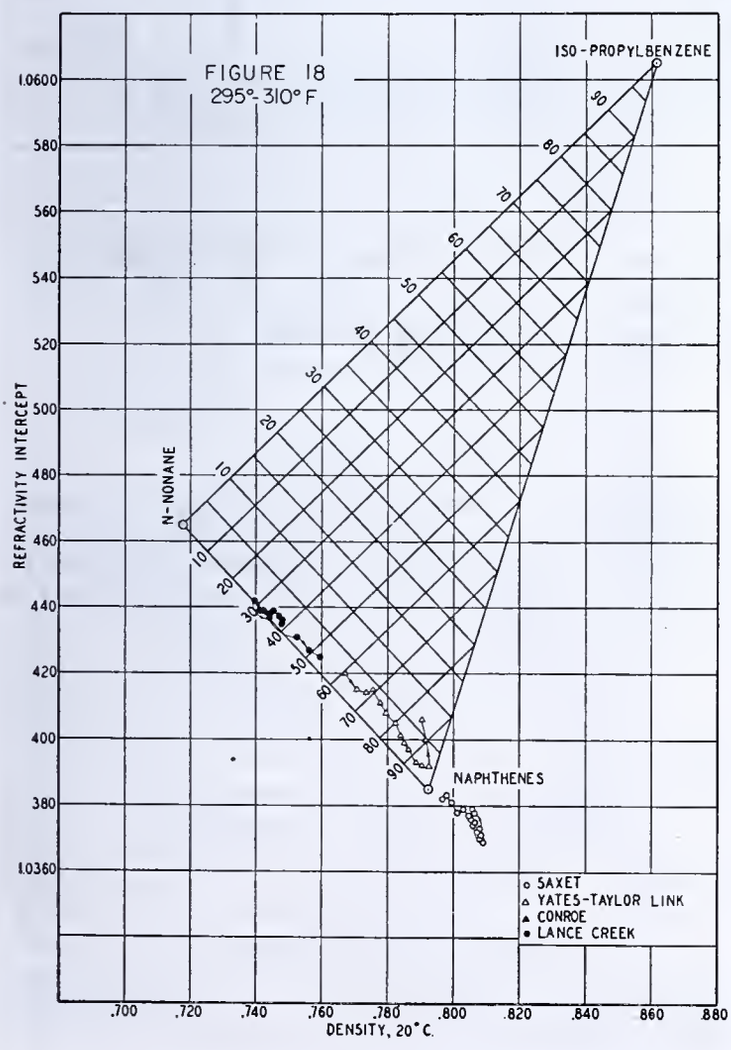
other data shown previously but do indicate that the points selected for this range are reasonably accurate considering the present knowledge of petroleum naphthas in this boiling range. The paraffin point is based on the average of the constants for 2,6-dimethyloctane, 3,5-dimethyloctane, 2,5-dimethyloctane, and 2,7-dimethyloctane. The naphthene point is defined by the constants for *n*-propylcyclohexane and *n*-butylcyclopentane.

No data for the Conroe naphtha are shown in Figure 18, since the distillation of this sample was discontinued at 295° F. For the sake of clarity, not all data obtained in the column distillations are shown in Figures 4 to 19, as many of the points would have coincided and overlapped. The data omitted would not have altered the general shape or extent of any of the curves but, on the contrary, would have strengthened the conclusions drawn. The small directional arrows indicated on the curves show the increase in boiling point of the fractions. The fact that some of the points shown on Figure 18 fall beyond the boundaries of the triangle has several implications: (1) the literature values of the constants for some of the pure compounds are in error; (2) undue weight was given to certain compounds in defining the shape of the particular triangle; or (3) certain naphthas will exhibit unusual characteristics not encountered in most cases. Unusual characteristics would include the appearance of significant quantities of bi- or dicyclic naphthenic compounds in the higher-boiling portion of the naphtha.

Having determined the volume per cent of aromatics, naphthenes, and paraffins in each fraction, these volume per cents are converted to volume per cents based on the total sample which are summed progressively for each fraction at the corrected boiling points recorded for each of these fractions. The volume sum per cents and the recorded boiling points are plotted on suitable graph paper and curves are drawn through each of the points. From these curves the volume sum per cents of aromatics, naphthenes, and paraffins are read at each degree from 97° to 320° F. The difference between the volume sum per cents for any 2 successive degrees is the volume per cent of aromatics, naphthenes, or paraffins distilled throughout that specific degree. From these data, curves indicating the volume per cent per Fahrenheit degree of each of the hydrocarbon types are plotted as shown on Figure 20. These charts show the results of the analysis in graphical form. The amounts of the individual aromatics, and the total amounts of naphthenes and paraffins have been determined from the data obtained, and the amounts of individual naphthenes and paraffins, or any desired group of compounds, may be estimated from the curves shown on Figure 20. Further examination of the data obtained by these analyses will give the amounts of most of the individual hydrocarbons having boiling points below 243° F. Table V gives the amounts of the individual hydrocarbons boiling below 243° F. in



10 representative naphthas as determined by the procedure outlined in the following paragraphs. A paper in preparation will give additional analyses together with some correlations that have been obtained from a study of the data. In general, the data will substantiate most of the conclusions drawn by Forziati, Willingham, Mair, and Rossini (*3*). Unfortunately, two of the samples indicated in Table V, Saxet and Wade City, were prepared by the refineries in such a manner that the total quantity of hexanes naturally occurring in the crude oils was not retained in the naphthas. This was indicated by the Engler distillation initial boiling points of the samples which were 170° and 162° F., respectively. A study of similar analyses reported by other laboratories indicates that in some cases a similar deficiency must have existed in the samples analyzed.



PROCEDURE FOR ESTIMATING INDIVIDUAL HYDROCARBONS IN TEMPERATURE RANGE 97° TO 243° F.

The estimations will not include any material having boiling points below *n*-pentane, and *n*-pentane will be indicated only in such amount as occurs in a mixture with higher-boiling material. Therefore the first fraction considered in this procedure is that fraction whose boiling point shows a decided rise, 5° or more, above the boiling point of *n*-pentane at about 97° F. An increase in the refractive index for the sodium D line (n_D^{20}) will accompany this temperature rise. This increase will reach a peak at about 121° F. and then decrease until the reading is 1.3750 or lower, at approximately 136° F. From 97° to 136° F. the material present in the fractions will consist of cyclopentane and *n*-pentane, 2,2-dimethylbutane, and 2,3-dimethylbutane. The total quantity of cyclopentane and 2,2-dimethylbutane is determined by the equal-area method of division from a plot of temperature against volume per cent distilled from 97° to 136° F. The difference between this quantity and the amount of cyclopentane determined from Figure 4 is the amount of 2,2-dimethylbutane in the sample.

From the point where the refractive index recedes to 1.3750 at about 136° F. to the point where the index and the density reach a minimum due to a maximum concentration of 2-methylpentane at about 141° F., all the material in the fractions will be 2,3-dimethylbutane and 2-methylpentane. The percentage of 2,3-dimethylbutane is calculated from the equation

$$0.0036Y = n_D^{20} - 1.3714 \qquad (6)$$

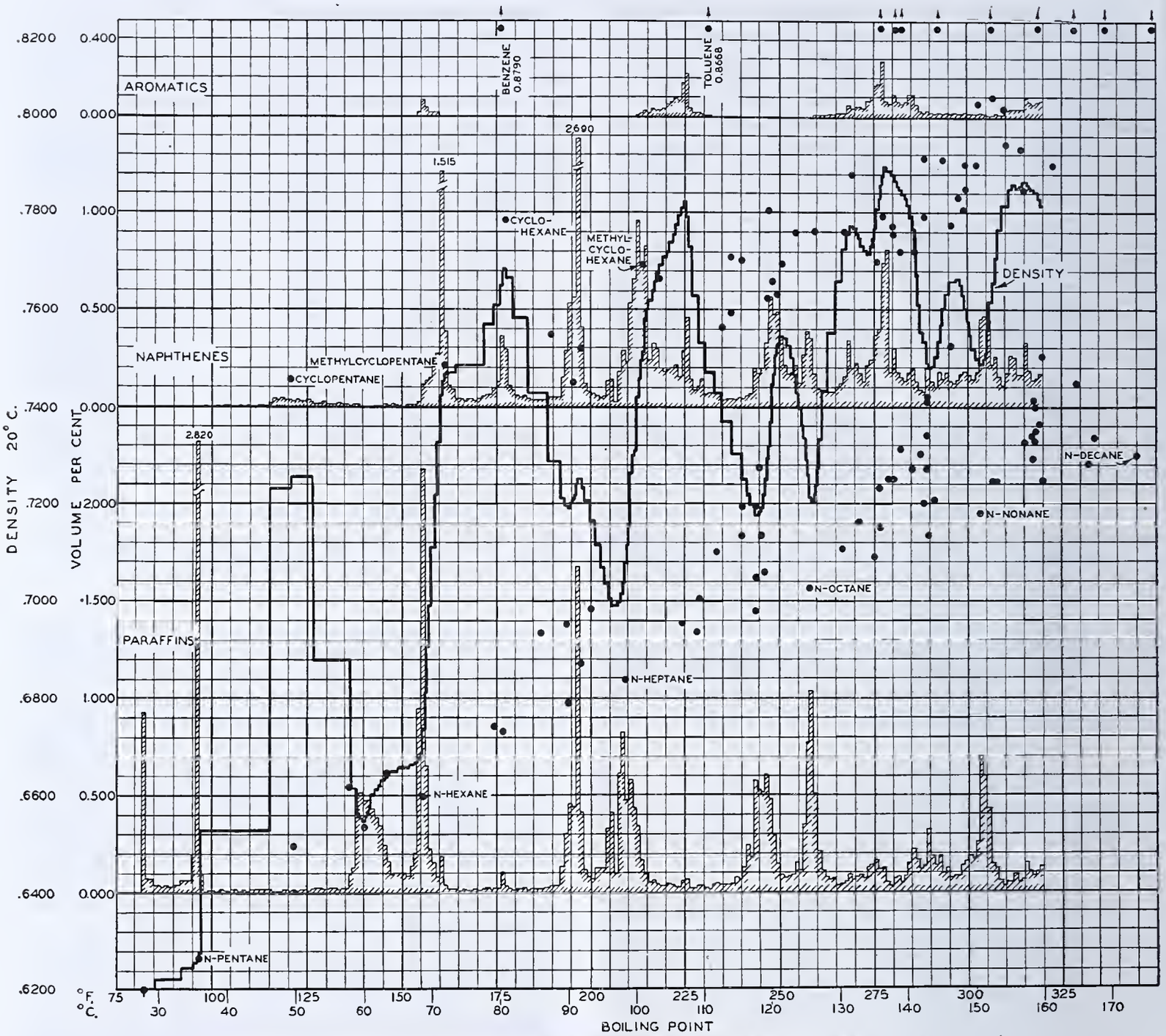


Figure 20. Volume Per Cent per Degree Fahrenheit, Paraffins, Naphthenes, and Aromatics

where (100)Y = volume per cent of 2,3-dimethylbutane and n_D^{20} is the refractive index of the fraction. If there are several fractions in this minimum region, Equation 6 is used to the middle of the region.

From this minimum to about 145° F. the material is 2-methylpentane and 3-methylpentane, and the amount of 2-methylpentane is determined by use of the equation

$$0.0051W = 1.3765 - n_D^{20} \qquad (7)$$

where (100)W is the volume per cent of 2-methylpentane. Equation 7 is used up to and including that fraction showing a "low" in the volume per cent of 2-methylpentane indicated by the equation, which occurs at about 145° F. An increase in the amount of 2-methylpentane shown by the use of the equation indicates that n-hexane is responsible for this increase.

From the above "low" in the amount of 2-methylpentane calculated to a temperature of 150° F., the fractions usually contain only 3-methylpentane and n-hexane, the amount of n-hexane being calculated by use of the equation

$$0.0016Z = 1.3765 - n_D^{20} \qquad (8)$$

where (100)Z is the volume per cent of n-hexane. In a few cases some benzene will be present in fractions distilling between 146° and 150° F. and the resolution of the fractions is then made by simultaneous solution of the two following equations, based on refractive index and density:

$$A = 0.0128B + \frac{n_D^{20} - 1.3765}{0.1246} \qquad (9)$$

and

$$0.4392B = (43.8163)(H) + \frac{0.6643 - d_f}{0.0049} \qquad (10)$$

Equation 10 may be expressed as

$$B = (99.7639)(H) + \frac{0.6643 - d_f}{0.00215} \qquad (11)$$

In Equations 9, 10, and 11 the volume per cent of benzene is (100)A, of n-hexane is (100)B, and of 3-methylpentane is (100) ×

$(1 - A - B)$. H is from Equation 9 and is $\left(\frac{n_D^{20} - 1.3765}{0.1246}\right)$. All material in the temperature range from 150° to 167° F. will be *n*-hexane, methylcyclopentane, and benzene, if benzene is present in the sample. Methylcylcopentane is determined in the usual manner by use of the triangular graph for this temperature range. The base of this triangle is shown in Figure 5. Benzene is determined by the use of the specific dispersion equations, and by difference the remainder of the material in the fraction is *n*-hexane.

All material in the range 167° to 185° F. is cyclohexane, 2,2-dimethylpentane, 2,4-dimethylpentane, and benzene, if present. No differentiation is made between the 2 dimethylpentanes, cyclohexane is determined by the use of Figure 6, and benzene by the specific dispersion equations.

No aromatics occur in the fractions having boiling points between 185° and 203° F. The total amounts of naphthenes and paraffins in the range are determined from the use of Figure 7. All the naphthenic material in the fractions boiling between 185° and 191.5° F. is 1,1-dimethylcyclopentane. All paraffinic material within the range 185° to 196.5° F. is a mixture of 2,3-dimethylpentane and 2-methylhexane and is reported as such. From 191.5° to 196.5° F. the naphthenic component is *trans*-1,3-dimethylcyclopentane. The paraffinic material distilling between 196.5° and 203° F. is 3-methylhexane and the naphthenic material in this range is *trans*-1,2-dimethylcyclopentane. If Figure 7 indicates that the first fraction in this range from 185° to 203° F. still contains cyclohexane as the predominant naphthene, the naphthene material should be recorded as cyclohexane instead of 1,1-dimethylcyclopentane.

The triangular graph constructed on the base shown in Figure 8 is used for the determination of the amount of methylcyclohexane in the sample and is used from 203° to 215° F. Toluene is calculated from specific dispersions, and *n*-heptane is obtained by difference.

The graph for 215° to 222° F. indicates the amount of ethylcyclopentane in this temperature range. Toluene is calculated from specific dispersions, and the remainder of the material in the fractions is 2,2-dimethylhexane. (The amount of ethylcyclopentane indicated may include some other naphthenic material, which, if present, is probably a trimethylcyclopentane.)

The naphthenic material in boiling range from 222° to 235° F. is determined from Figure 11 and is recorded as a trimethylcyclopentane mixture. Two or more of these compounds may be present in these fractions. Toluene again is determined by specific dispersion measurements. The balance of the material in the fractions having boiling points between 222° and 226.3° F. is 2,2-dimethylhexane, and between 226.3 and 235° F. the paraffin constituents are 2,5-dimethylhexane and 2,4-dimethylhexane.

The graph for the temperature range 235° to 243° F. is used for the determination of what probably is a trimethylcyclopentane in this region. Toluene, if present, is calculated from specific dispersions, and remaining material is 2,3-dimethylhexane.

A technical aide without a knowledge of petroleum hydrocarbons and their behavior when distilled from petroleum naphthas can follow the above procedure for estimating the amounts of the individual hydrocarbons in a naphtha, but for greater accuracy a skilled analyst should check and complement the procedure outlined by the use of other combinations of data obtained in these distillations. The accuracy of the method increases with fractionating efficiencies and with larger charges of sample to the columns. Fractionating efficiency can be increased effectively by making the product take-off rates 2 to 5 ml. per hour instead of the rates that had to be used in these studies.

In these laboratories the distillations of the naphthas in the fractionating columns require approximately 50 hours for each naphtha. The total time required for the distillation, the analytical determinations, the calculations, and the plotting is from 80 to 100 hours. The distillations and analytical determinations require the services of technical personnel, whereas the calculations and plotting can be done by high-school students with a minimum of supervision.

ACCURACY OF THE METHOD

The accuracy of the determination of the aromatics by the use of specific dispersions is discussed by Thorne, Murphy, and Ball (*8*), and has been checked by analysis of synthetic mixtures containing known amounts of the individual aromatic compounds. When the aromatic content of a normal virgin naphtha is determined by this method, the reported result for any single aromatic compound will have a probable error of about ±0.2%. This probable error was checked by the analyses of the synthetic mixtures.

The accuracy of the determination of the naphthene and paraffin content of a naphtha has not been checked experimentally but from factors that can be estimated, it is believed that the determination of these two types will be within 10% of the reported result for that portion of the naphtha boiling below 243° F., and within 25% for that portion between 243° and 320° F. The greatest source of error below 243° F. is the nonadditivity of mixtures of aromatics with naphthenes and with paraffins, while the greatest source of error above 243° F. seems to be in the lack of knowledge as to the constitution of this portion of the naphtha.

For the estimations of individual naphthene and paraffin hydrocarbons it is felt that the estimations are correct to ±0.3% by volume of the total amount of sample charged to the columns when the volume per cent of the individual hydrocarbon is below 3% of the charge. When the amount is more than 3%, the accuracy of the estimate should be within ±10% of the amount reported. While overlapping of compounds into adjacent fractions occurs at the temperature divisions used in the scheme of analysis due to the holdup of the columns and a lack of sufficient fractionating efficiency, such overlapping is compensatory and should not be enough to cause errors exceeding the above limits.

The possible effects of the presence of sulfur compounds on the determinations are discussed in a Bureau of Mines report by Ball and Thorne (*1*). The conclusion reached is that 0.2% by weight of sulfur in a naphtha will not affect the final results of analyses for the three hydrocarbon types. Another of this series of papers by the Bureau of Mines (*7*) discusses a rapid method of analyzing naphthas for benzene, toluene, and seven-carbon-atom naphthenes by the use of specific dispersions and refractivity intercept–density diagrams.

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Determination of Total Solids in Resin Solutions

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Total solids in solutions of heat-stable resins in volatile solvents can be determined rapidly and with an average accuracy of 0.3% by first dissolving samples in a suitable nonvolatile, high-boiling solvent and then heating them in vacuo at 100° C. for 20 to 30 minutes while they are being agitated. If the proper high-boiling solvent were selected, this general method could be extended to the determination of any nonvolatile stable material in admixture with any volatile substance.

A NEW method for the routine determination of total solids in resin solutions has been developed, which is applicable to solutions of all resins, provided the resins do not change in molecular weight through the evolution of gases or moisture on heating. This "solution-evacuation" method consists essentially of dissolving a sample of resin solution in a high-boiling solvent, heating the resulting solution under vacuum to remove only the solvent in which the resin was dissolved originally, but not the high-boiling solvent, and observing the loss in weight of the system. The solution-evacuation method is much more rapid and accurate than any of the usual methods which depend upon heating at atmospheric pressure, either alone (*1*, *2*) or after the addition of a suitable high-boiling liquid (*4*, *5*, *6*), or upon heating in vacuo (*3*).

As a possible means of determining total solids, the measurement of several physical constants of resin solutions was considered. Among these, specific gravity and refractive index seemed most likely to give a useful method, but proved to be impractical. The high viscosity of the solutions made the determination of specific gravity with a Westphal balance practically impossible and the filling of a pycnometer a long and difficult task. The correlation between refractive index and total solids was only approximate and offered no promise of giving the necessary accuracy.

The resins from which solutions were prepared were definitely acidic; consequently, the acid numbers of their solutions possibly could give a measure of the total solids. However, the acid number could not be measured with the desired accuracy without very careful work. The acid number of the resins was about 60, that of the solutions was about 45. Thus, an error of one unit in the measurement of the acid number corresponds to a variation in the total solids of nearly 2%. Furthermore, usual small variations in the acid number of the solid resin also would cause an error in this method.

Direct methods also were considered. Evaporation of the solvent in a vacuum oven offered only a slight advantage over an air oven, because of poor conduction of heat to the sample.

The solution-evacuation method, like the direct methods, involves separating the solvent from the solid resin. However, in order to accomplish this rapidly and completely, without entrainment as occurs in the oven method, the sample of resin solution must be dissolved in dibutyl phthalate. This technique keeps the system, from which the solvent is being removed, liquid throughout the determination. The solution in dibutyl phthalate, contained in a small Erlenmeyer flask, is heated and evacuated, and its loss in weight determined. This loss in weight is the weight of the volatile solvent. Thus, the per cent total solids of the sample is calculated readily. Such determinations require 35 minutes to 1 hour. The average accuracy and precision of the results, expressed as per cent total solids, are 0.2%.

In order to remove the volatile solvent completely from the solution and at the same time avoid mechanical loss of any part of the sample, it was found necessary to provide some surface at which the bubbles of solvent vapor could readily form. This was accomplished by placing about 6 polished steel balls in the flask and slowly rocking it.

Although the solution-evacuation method has been applied only to solutions of resins in nonaqueous solvents, it is probable that its use could be extended to water solutions if one of the commercially available polyethylene glycols were used to replace the dibutyl phthalate. In fact, by adding a suitable high-boiling solvent to the sample, this method could be applied in the determination of nonvolatile stable material in admixture with any volatile substance.

APPARATUS

An apparatus was designed to rock two flasks in an oil bath in such a manner that the axis of rotation passes through the base of the flasks. This arrangement prevents any unnecessary motion of the flasks and attendant splashing of the hot oil. The power for this rocking is supplied by a small electric motor with a reduction gear assembly. (The motor used in this investigation was a Ratiomotor, Type MB58328, manufactured by the Boston Gear Works, Philadelphia, Pa.) The rocking arm is attached to an eccentric on the motor by means of a connecting rod. The built-in reduction gear on this motor gives the driving shaft a speed of 50 r.p.m. The frame for holding the flasks was designed to allow the flasks to be raised from and lowered into the oil bath. Figure 1 shows the construction of the rocking device.

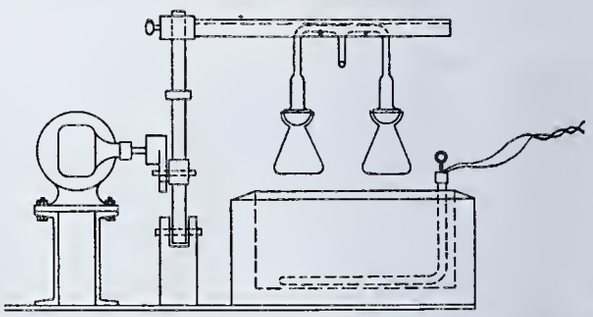

Figure 1. Construction Details of Rocking Device

The oil bath is constructed of sheet steel and is lagged with a 1.25-cm. (0.5-inch) layer of 85% magnesia. The bath liquid is heated with a 200-watt tubular immersion heater bent and placed to deliver the heat near the bottom of the bath. The heat input is controlled by a small (5-ampere) Variac.

Fifty-milliliter Erlenmeyer flasks fitted with 25/15 spherical joints are attached by means of clamps to a two-outlet manifold also fitted with spherical joints each of the same size. This manifold is attached to the rocking device and is connected by means of heavy-walled rubber tubing to a manometer and two solvent traps of conventional design. The two solvent traps are connected in series and are cooled in a mixture of dry ice and Cellosolve. The system is evacuated by means of a Hy-vac oil pump. The vacuum system also contains a needle valve which opens to the atmosphere and is connected to the system between the manometer and flasks. This valve permits the control of the pressure in the flasks by bleeding air into the system. Figure 2 shows the relationships of the various parts of the apparatus.

REAGENT

Any good grade of dibutyl phthalate may be used in this determination. All the grades tested contained small amounts of volatile matter. It is highly advantageous to reduce the amount of volatile matter from its normal value of 23 to 25 mg. per 10 ml. to about 5 mg. per 10 ml. by sparging it with dry air for 48 hours.

PROCEDURE

Place about 6 steel balls, 0.77 cm. ($^5/_{16}$ inch) in diameter, in a 50-ml. Erlenmeyer flask, add 10 ml. of dibutyl phthalate, and weigh (W_1). Add 2 to 3 ml. of the resin solution, using a 5-ml. pipet with the tip cut off, and taking care to get all the resin solution into the dibutyl phthalate with none of the resin on the side of the flask or the joint. This operation is made easier if the solution adhering to the outside of the pipet is removed with a paper towel or other suitable material before the sample is delivered to the flask. Weigh the flask after adding the resin solution (W_2). Then, $W_2 - W_1$ represents the weight of the sample. All weighings are made to the nearest milligram. A second flask is charged in the same way.

Set the flasks firmly in place on the apparatus and clamp them in position. Lower them into the bath, which is maintained at 100° ± 2° C. by means of the Variac. With the needle valve open, start the vacuum pump and set the rocking device in motion. By closing the needle valve, slowly reduce the pressure in the flasks at such a rate that moderate boiling of the solution is maintained. The full vacuum of the pump should be attained in 5 minutes. Continue the determination for the time required by the sample being examined.

Admit air to the apparatus by opening the needle valve; then stop the pump and rocking device. Remove the flasks and allow them to cool. Remove the adhering oil by rinsing the flasks with methyl acetate or other suitable solvent and wiping them carefully with tissue paper. Weigh the cooled flasks (W_3). Then, $W_3 - W_1$ is the weight of the solids. Run a blank to determine the loss in weight of the dibutyl phthalate.

$$\frac{(W_3 - W_1 + B) \times 100}{W_2 - W_1} = \% \text{ total solids}$$

where B represents the blank value for a particular lot of dibutyl phthalate.

APPLICATION OF THE METHOD

In order to establish the proper conditions for the determination of total solids of a variety of resin solutions, as well as to ascertain the practical accuracy of the method, a series of solutions of known total solids content was prepared and analyzed.

A weighed amount of Flexalyn (diethylene glycol ester of rosin, registered in U. S. Patent Office by Hercules Powder Company) was dissolved in sufficient 50 D solvent (principally xylenes) to give approximately an 80% solution. The resin was dissolved by heating under a reflux condenser. After being cooled, the solution was weighed and its actual solids content was calculated. The total solids in this solution was determined experimentally by the foregoing procedure. The sample sizes usually were between 1.5 and 2.0 grams and the final pressure was 5 mm. or less. The effects of time of heating, temperature of the oil bath, and variations in sample size were studied.

The results of these experiments (Table I) indicate that correct results are obtained when the solutions are heated for 45 minutes at 100° C. or for 30 minutes at 110° C.

Solutions of Petrex 5 (a glycol ester of a terpene–maleic anhydride adduct, registered in U. S. Patent Office by Hercules Powder Company), a hard resin, in ethyl acetate, ethyl alcohol, methyl ethyl ketone, and toluene were prepared in the manner described for Flexalyn solutions. Since the Petrex 5 itself contained volatile substances, the solid resin was first analyzed by the solution-evacuation procedure and found to lose 0.8% of its weight. This value was used as a correction factor in calculating compositions of solutions of Petrex 5 in the various solvents.

Table I. Total Solids of Flexalyn Solutions in 50 D Solvent (Chiefly Xylenes)

Time of Heating under Vacuum	Temperature of Bath	Total Solids in Flexalyn Solution		
		Calcd	Found	Average found
Min.	° *C.*	%	%	%
20	100 ± 1	79.9	80.8, 81.1	81.0
30	100 ± 1	79.9	80.9, 80.7 86.8[a], 80.7	80.8
45[b]	100 ± 1	79.9	80.1, 79.6 79.7, 79.7 80.1, 80.2	79.9
20	110 ± 2	79.9	80.9, 81.0 80.3, 80.3	80.6
30[c]	110 ± 2	79.9	79.8, 79.7 79.7, 80.0 80.3, 80.3	80.0

[a] Arbitrarily rejected in computing average.
[b] Sample sizes ranged from 1.3 to 2.9 grams.
[c] Sample sizes ranged from 1.3 to 3.7 grams. High values, 80.3 and 80.3, were obtained on samples of 3.65 and 3.67 grams.

Table II. Total Solids of Petrex 5 in Various Solvents

Sample Weight	Time of Heating under Vacuum	Temperature of Bath	Total Solids in Solution	
			Calcd.	Found
Grams	*Min.*	° *C.*	%	%
		In Ethyl Acetate		
2.195	30	100	73.6	73.8
2.160	30	100	73.6	73.8
2.236	20	100	73.6	73.7
2.309	20	100	73.6	73.5
2.312	20	110	73.6	73.7
2.194	20	110	73.6	73.6
		In Ethyl Alcohol		
1.770	30	100	74.4	74.9
2.008	30	100	74.4	74.8
1.867	30	100	74.4	75.0
2.480	30	100	74.4	74.9
2.112	20	100	74.4	74.9
2.183	20	100	74.4	74.9
2.278	15	110	74.4	74.5
2.485	15	110	74.4	74.8
		In Methyl Ethyl Ketone		
1.993	30	100	74.6	74.5
2.059	30	100	74.6	74.6
2.030	20	100	74.6	74.8
2.359	20	100	74.6	74.7
2.156	20	110	74.6	74.5
2.323	20	110	74.6	74.6
		In Toluene		
2.108	30	100	74.1	74.0
2.500	30	100	74.1	73.6
1.894	30	100	74.1	73.7
2.746	30	100	74.1	73.4
2.042	20	100	74.1	73.7
2.841	20	100	74.1	73.8
3.181	15	110	74.1	73.7
3.116	15	110	74.1	73.8

The results presented in Table II show that the solvent can be completely removed from these solutions at either 100° or 110° C. by heating for 20 minutes or longer. The values for Petrex 5 in ethyl alcohol are slightly high. It may be that in preparing the solution by heating the resin, which is appreciably acidic, a small amount of the alcohol may have been esterified.

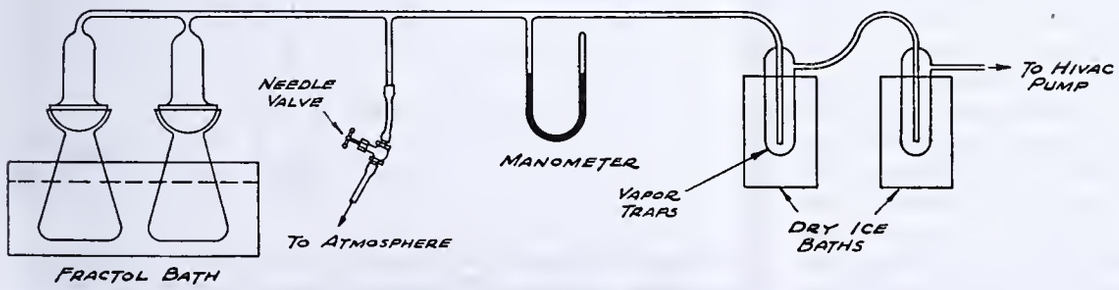

Figure 2. Complete Assembly of Apparatus

Solutions of Petrex 7HT (a glycol ester of a terpene–maleic anhydride adduct, registered in U. S. Patent Office by Hercules Powder Company), a soft resin, in ethyl acetate, ethyl alcohol, methyl ethyl ketone, and "mixed solvent" (chiefly toluene) were prepared as described for Flexalyn. The solid resin, when analyzed by the solution-evacuation procedure, lost 1.60% of its weight. This value was used as a correction factor in calculating the compositions of the

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Table III. Total Solids of Petrex 7HT in Various Solvents

Sample Weight	Time of Heating under Vacuum	Temperature of Bath	Total Solids in Solutions	
			Calcd.	Found
Grams	*Min.*	*° C.*	%	%
		In Ethyl Acetate		
2.226	30	100	73.3	73.5
2.134	30	100	73.3	73.5
2.233	20	100	73.3	73.8
2.328	20	100	73.3	73.8
2.245	15	110	73.3	73.4
2.272	15	110	73.3	73.3
		In Ethyl Alcohol		
2.023	45	100	74.3	74.8
2.263	45	100	74.3	74.7
2.074	30	100	74.3	74.9
1.980	30	100	74.3	75.0
2.221	30	110	74.3	75.0
2.252	30	110	74.3	74.8
2.229	15	110	74.3	75.3
2.026	15	110	74.3	75.3
		In Methyl Ethyl Ketone		
2.350	30	100	74.4	74.5
2.159	30	100	74.4	74.5
2.532	20	100	74.4	74.6
2.162	20	100	74.4	74.7
1.998	15	110	74.4	74.3
2.459	15	110	74.4	74.3
		In Mixed Solvent (Chiefly Toluene)		
1.756	30	100	73.5	73.3
1.932	30	100	73.5	73.3
2.963	30	100	73.5	73.4
2.540	30	100	73.5	73.3
2.320	20	100	73.5	73.3
2.396	20	100	73.5	72.9
2.651	20	100	73.5	73.4
2.711	20	100	73.5	73.4
2.399	15	100	73.5	73.7
2.601	15	100	73.5	73.3

solutions of Petrex 7HT in the various solvents. These solutions were analyzed by the solution-evacuation method.

The data obtained on these solutions (Table III) are similar to those obtained with the other solutions. Again the slightly high values obtained when using alcohol as solvent may be explained on the basis of esterification.

The data recorded in Tables II and III are all consecutive results; none has been discarded. They indicate that analyses by this procedure, expressed as per cent total solids, have an average accuracy and precision of 0.2%.

SUMMARY

This paper describes a new technique for the determination of the total solids in solutions of synthetic resins in nonaqueous solvents, which has been used as a routine control method for nearly two years. Results of higher accuracy are obtained more rapidly than by any method previously used. It is possible that this method can be extended to aqueous solutions by using one of the polyethylene glycols in place of dibutyl phthalate.

ACKNOWLEDGMENT

The authors wish to acknowledge the assistance of Marie C. Barry, who performed most of the analyses for this paper.

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Quantitative Determination of Phenolic Fungicides

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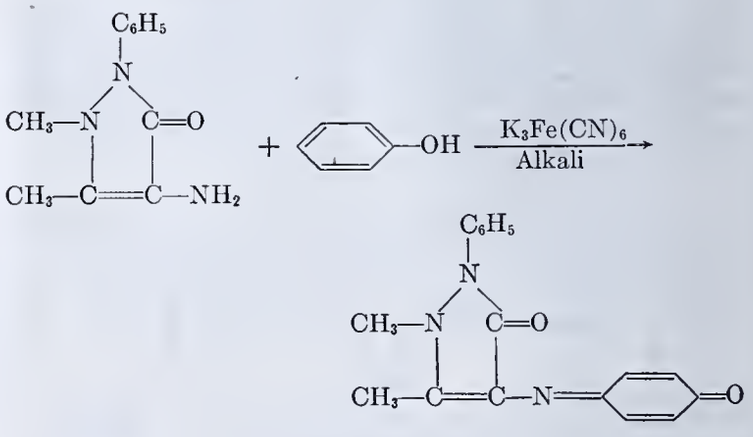
The color reaction of 4-aminoantipyrine with the textile mildew preventive 2,2′-methylenebis[4-chlorophenol] in the presence of potassium ferricyanide and dilute sodium carbonate has been found adaptable to quantitative analysis for this phenolic material and has been used for its determination in fabric. Absorption curves and standard calibration curves are given for several other commercially important phenols, with the suggestion that this color reaction may find application in the quantitative determination of many phenolic fungicides, germicides, and other materials.

RECENTLY certain fungicidal materials have come into extensive use as mildew preventives for fabrics destined to be used in tropical areas (*4*). One of the compounds which has been most widely used for this purpose is 2,2′-methylenebis[4-chlorophenol], known to the textile trade as Compound G-4. The method most commonly used in the past for the determination of this compound on fabric is a modification of the halogen microprocedure of Willard and Thompson (*5*). This method, however, has been found in practice to be time-consuming and the results to be uncertain in some cases because of the presence of inorganic or organically bound chlorine in other forms on the fabric. Both these difficulties are avoided by the method here described. Preliminary data on several additional phenolic compounds suggest that the new procedure may be useful in the determination of phenolic fungicides, germicides, and other materials.

The reaction used is based on the observation made by Emerson that 4-aminoantipyrine condenses with aromatic amines (*1*) in the presence of acid oxidizing agents and with phenols (*2*, *3*) in the presence of alkaline oxidizing agents to yield a series of antipyrine dyes. On the basis of this reaction Emerson proposed a color test for phenols (*2*). The structure suggested for the dyes produced in the reaction is shown in the following example for phenol:



Emerson concluded that substitution took place in the position para to the phenolic OH group and that the structural requirements for the reaction were as follows:

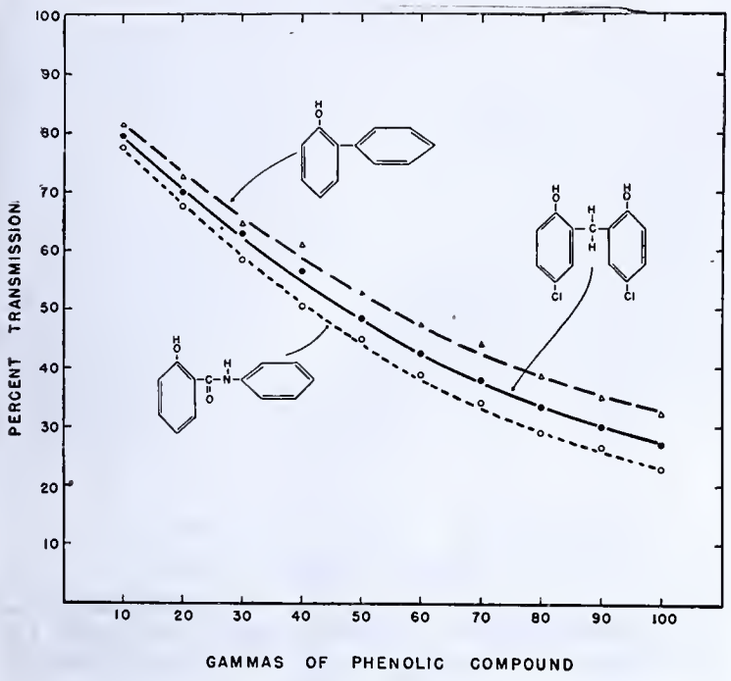


Figure 1. Standard Calibration Curves for Dyes Produced by Reaction of 4-Aminoantipyrine with o-Phenylphenol, 2,2'-Methylenebis[4-chlorophenol], and Salicylanilide

Filters used for data in Figures 1, 2, and 3 are those suggested in text where reactions of various compounds with 4-aminoantipyrine are described

1. At least one free phenolic group must be present.
2. The position para to the phenolic OH must either be unsubstituted, or substituted by halogen, carboxyl, sulfonic acid, hydroxyl, or methoxyl, which groups are expelled in the reaction. Substitution in the para position by alkyl, aryl, nitro, benzoyl, nitroso, or aldehyde groups blocks the reaction.

Under certain conditions the reaction of 4-aminoantipyrine with phenols is readily amenable to quantitative treatment in the determination of phenolic fungicides. A method for the determination of 2,2'-methylenebis[4-chlorophenol] on fabric is presented below and absorption and standard calibration curves are given for *o*-phenylphenol, salicylanilide, 2,2'-methylenebis-[3,4,6-trichlorophenol], 4,4'-isopropylidenediphenol, 4,4'-isopropylidenebis[2-chlorophenol], 2,4-dichlorophenol, pentachlorophenol, and tetrabromo-*o*-cresol.

DETERMINATION OF 2,2'-METHYLENEBIS[4-CHLOROPHENOL]

This compound reacts with 4-aminoantipyrine in an alkaline oxidizing medium to form a red dye. Under the conditions described below the dye develops almost immediately and is relatively stable for several hours. The standard calibration curve, made with an Aminco Type F photometer, balanced against distilled water, and the absorption curve, taken with a Coleman Model 10S spectrophotometer, are shown in Figures 1 and 4, respectively. The procedure for determination of this compound in fabric is presented below.

Solutions. 4-Aminoantipyrine, 2%. Two grams of 4-aminoantipyrine (m.p. 108–109°) dissolved in 100 cc. of distilled water. This compound is made from antipyrine by the method described by Emerson (*1*). It has been kept in solution in stoppered dark bottles in the laboratory for several months without noticeable deterioration.

Potassium ferricyanide, 8%. Eight grams of C.P. potassium ferricyanide dissolved in 100 cc. of distilled water.

Sodium carbonate, 0.025%. C.P. anhydrous sodium carbonate (4.5 grams) dissolved in 18 liters of distilled water. The pH of this solution should be between 10.4 and 10.6.

Standard Curve. Dissolve 200 mg. of 2,2'-methylenebis-[4-chlorophenol] in 100 cc. of acetone in a volumetric flask, place 1 cc. of this solution in a second 100-cc. volumetric flask, and dilute to the mark with sodium carbonate solution. This solution, containing 20 micrograms of G-4 per cc. is used to obtain suitable aliquots covering the range of 20 to 100 micrograms. Place each aliquot in a 25-cc. volumetric flask and add 0.5 cc. of aminoantipyrine reagent. Dilute to the mark with the sodium carbonate solution, add 0.25 cc. of 8% potassium ferricyanide, and shake vigorously. After 5 minutes, pour the solution into a colorimeter tube and measure the color, using a suitable filter (green, about 500 millimicrons). The same grade of product should be used in making up the standard curve as was applied to the cloth sample in question.

Fabric Analysis. For samples of cloth containing up to 2% of compound G-4, weigh a 1-gram sample of the very finely cut material to the nearest milligram, and place the sample in a 200-cc. beaker. Add 50 cc. of 0.25% sodium carbonate solution and heat to gentle boiling. Boil gently for 5 minutes. Pour the hot solution off the fabric into a 200-cc. volumetric flask (Pyrex). Repeat the extraction twice with 50 cc. of the sodium carbonate solution, each time boiling for 5 minutes and combining the extracts with the first one in the volumetric flask. Dilute the solution to near the mark with the sodium carbonate solution, using the diluent to wash the textile material twice. Cool the flask to room temperature and dilute exactly to the mark. Filter about 20 cc. through a dry filter and use the proper aliquot of this solution (usually 1 to 2 cc.) to get a reading on the standard curve. Place the aliquot in a 25-cc. volumetric flask and add 0.5 cc. of 2% 4-aminoantipyrine solution. Dilute to the mark with 0.025% sodium carbonate solution, add 0.25 cc. of 8% potassium ferricyanide solution, shake vigorously, and after 5 minutes pour into a colorimeter tube and measure the color. After mixing the re-

Table I. Comparative Determination of 2,2'-Methylenebis[4-chlorophenol] (Compound G-4) on Fabric

(By the Willard and Thompson halogen microprocedure and the 4-aminoantipyrine method. The replicates in the aminoantipyrine method are different individual samples cut from a single piece of cloth.)

Sample No.	Per Cent on Fabric: Aminoantipyrine method	Per Cent on Fabric: Willard-Thompson method
1	0.89	0.99
	0.91	
	0.88	
	0.87	
2	1.76	1.75
	1.73	
	1.76	
	1.74	
3	1.10	1.07
	1.05	
	1.07	
	1.06	
4	1.68	1.69
	1.67	
	1.64	
	1.68	

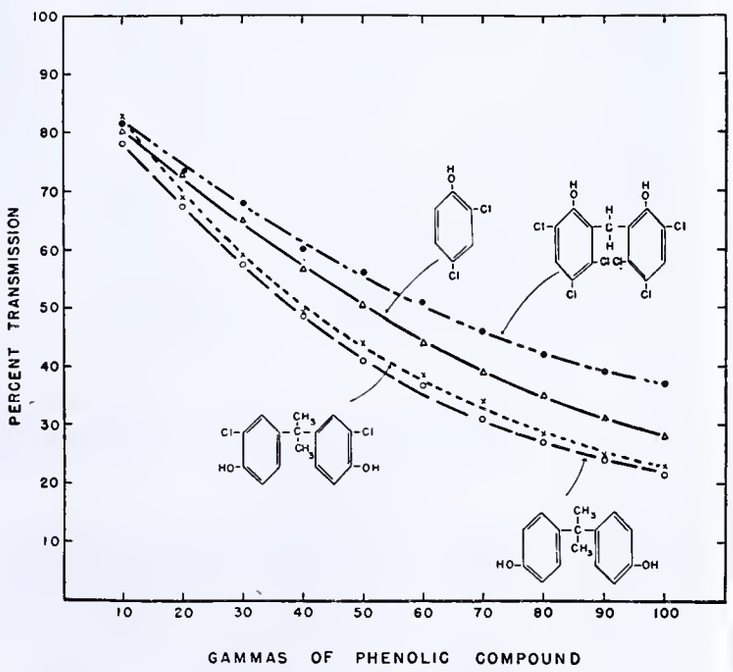


Figure 2. Standard Calibration Curves for Dyes Produced by Reaction of 4-Aminoantipyrine with 2,2'-Methylenebis[3,4,6-trichlorophenol], 4,4'-Isopropylidenediphenol, 4,4'-Isopropylidenebis[2-chlorophenol], and 2,4-dichlorophenol

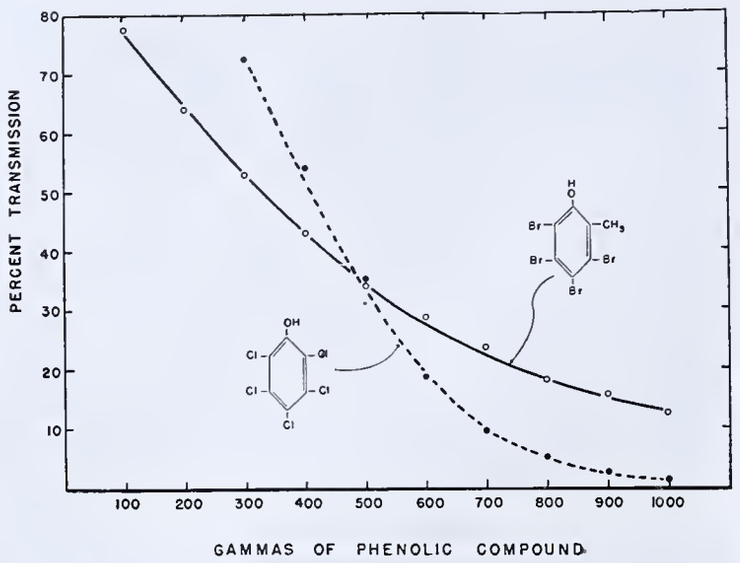


Figure 3. Standard Calibration Curves for Dyes Produced by Reaction of 4-Aminoantipyrine with Pentachlorophenol and Tetrabromo-o-cresol

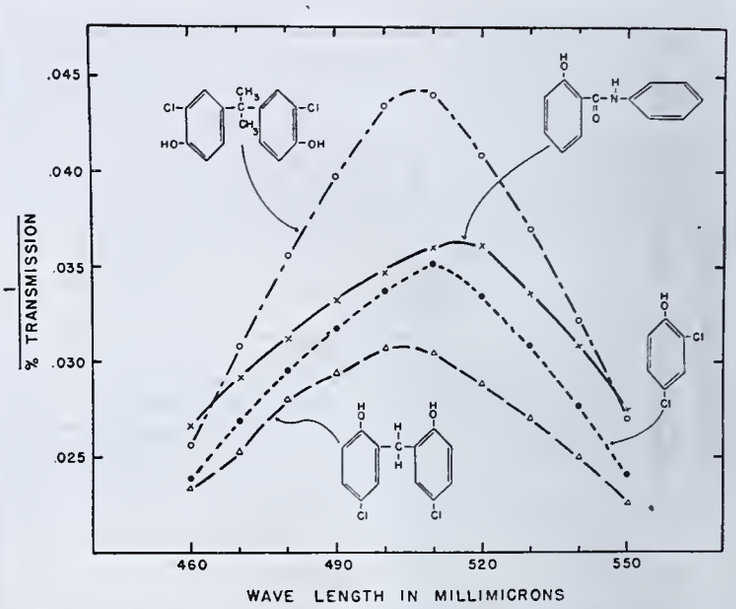


Figure 4. Absorption Curves for Dyes Produced by Reaction of 4-Aminoantipyrine with 2,2'-Methylenebis[4-chlorophenol], 2,4-Dichlorophenol, Salicylanilide, and 4,4'-Isopropylidenebis[2-chlorophenol]

100 micrograms of phenolic compound used to develop color

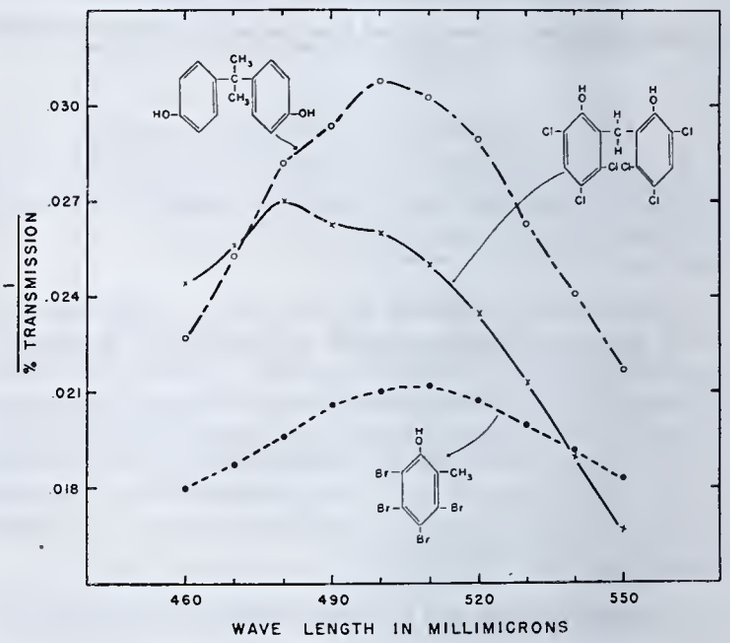


Figure 5. Absorption Curves for Dyes Produced by Reaction of 4-Aminoantipyrine with 4,4'-Isopropylidenediphenol, 2,2'-Methylenebis[3,4,6-trichlorophenol], and Tetrabromo-o-cresol

For first two compounds 100 micrograms of each compound were used to develop color, as described in section on standard curves. For tetrabromo-o-cresol 400 micrograms of phenolic compound were used.

agents, the tubes should not be exposed to direct sunlight or strong artificial light, as this sometimes causes fading of the colors.

A comparison of results obtained on analyzing four samples of fabric by the Willard and Thompson method (chlorine determination) and the 4-aminoantipyrine method is given in Table I. It was found that the colorimetric determinations could be made on 16 samples of fabric in less than 2 hours, including weighing of samples, extraction, development, and measurement of color.

DATA ON OTHER PHENOLS

Absorption curves and standard calibration curves were made for a group of other phenols according to the methods described above for Compound G-4. The several standard calibration curves are shown in Figures 1, 2, and 3, and the absorption curves in Figures 4 and 5.

Salicylanilide, a fabric preservative known commercially as Shirlan, produces a red dye which fades very gradually; calibration and absorption curves are shown in Figures 1 and 4. *o*-Phenylphenol, an industrial preservative for casein paints, cosmetics, leather finishes, and sizing materials which is known commercially as Dowicide 1, produces a stable red dye with a calibration curve as shown in Figure 1. 2,2'-Methylenebis[3,4,6-trichlorophenol], a germicide for use in soaps, forms a stable red color with calibration and absorption curves as shown in Figures 2 and 5. The large amount of inorganic chloride present is said to make the determination of this germicide in soap very difficult by the Willard and Thompson procedure. 4,4'-Isopropylidenediphenol, a material currently in the early stages of commercial development, forms a stable antipyrine dye, whose standard calibration and absorption curves are shown in Figures 2 and 5, respectively. 4,4'-Isopropylidenebis[2-chlorophenol], a chlorinated analog of the material previously mentioned, is claimed to be a potent fungicide and has been suggested as a fabric preservative. Standard calibration and absorption curves are shown in Figures 2 and 4.

2,4-Dichlorophenol, an intermediate in the manufacture of the weed-killer, 2,4-dichlorophenoxyacetic acid, forms a stable red color with standard calibration and absorption curves as shown in Figures 2 and 4.

Tetrabromo-*o*-cresol, used on a limited scale as a fabric preservative, reacts with 4-aminoantipyrine to produce a green color with an absorption maximum at 540 mμ, which slowly changes to a red color. After 1 hour the red color is fully developed and is stable for at least 12 hours thereafter. The standard calibration curve and absorption curve for the red color are given in Figures 3 and 5, respectively. Measurements were taken for both curves exactly 60 minutes after combination of the reagents.

Pentachlorophenol, a wood and fabric preservative known under the trade name of Dowicide 7, reacts with 4-aminoantipyrine to give a green color which gradually fades to colorless over a period of 2 hours. A satisfactory calibration curve could be obtained by measuring the color at 30 minutes after development of color, using a 640 filter (Figure 3). In this reaction it was found that 0.5 cc. of the potassium ferricyanide solution and 0.75 cc. of the aminoantipyrine solution gave the best development of color.

DISCUSSION

It can be seen from the data presented that 4-aminoantipyrine promises to be a very useful reagent for the quantitative estimation of phenols. The only element appearing to require special care in the analysis is the pH of the reaction medium. Deviations of 0.5 of a pH unit in either direction from the limits given caused changes in both the intensity and stability of the color produced. Attempts to utilize buffer mixtures were not successful, since the ions used in the buffer mixtures interfered with the development of the color. Thus boric acid–sodium hydroxide,

glycine–sodium hydroxide, and sodium tetraborate–sodium carbonate could not be used.

It will be noticed that the reactions of 4,4′-isopropylidenediphenol and 4,4′-isopropylidenebis[2-chlorophenol] represent an anomaly to the structural requirements for the reaction as reported by Emerson (*2*). Both of these compounds have an aryl alkyl substituent para to the phenolic OH group which supposedly would block the reaction since they could not be expelled by the ferricyanide oxidizing agent. The readiness with which these compounds react with 4-aminoantipyrine suggests that something other than a para substitution takes place and brings out the possibility that in cases where the para position is blocked ortho-quinoid structures may be formed. More work is needed to elucidate the exact nature of the reaction between phenols and 4-aminoantipyrine.

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Improved Method of Flame Photometry

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An improved flame photometer, employing a dual optical system, has been devised. With this instrument the internal standard principle often employed in spectral analysis may be used. Since by this method light intensity ratios are measured rather than absolute light intensities, the disturbing effects caused by gas and air pressure fluctuations, by the presence of foreign ions and molecules, and by viscosity differences are considerably reduced. The construction and performance of this photometer are discussed in detail.

IN A recent paper (*1*) a rapid analytical technique for the determination of sodium and potassium in aqueous solution was presented. An instrument known as the flame photometer was described, and the analytical procedure explained. Briefly, the method consists of atomizing an aqueous solution of the metal into the base of a gas burner, whereupon the vapor is carried into the flame and ignited. The light arising from the flame characteristic of the element being determined is filtered free of other radiation and is brought to fall upon a photocell. By measuring the intensity of the light produced with solutions of known concentration and preparing a calibration curve of intensity versus concentration, the metal content of other solutions may subsequently be determined by making use of the curve.

The prime requisite of successful flame photometry by this method is the establishment of constant atomization and burning conditions in the instrument. Considerable investigative work led to a design of instrument which would fulfill this condition with reasonable satisfaction. In the flame photometer previously described, these problems had been solved to obtain an average accuracy of ±3% of the total element present in the sample in a single determination. The errors made were found to be almost entirely random—i.e., equal number positive and negative—and it could be assumed that they came entirely from momentary variations of light intensity in the flame.

In the early course of this work, however, it was noted that an error far more serious in nature than this instrumental error could arise when excessive amounts of certain ions were introduced into solutions containing sodium or potassium—for example, the presence of 1% sulfuric acid in a solution containing 100 p.p.m. of sodium reduced the light emitted by the flame by some 15%. It was further noted that acids, salts, or indeed almost any foreign molecule, similarly reduce the apparent sodium content of solutions as analyzed by the flame photometer.

The most obvious manner in which to correct this type of error is to compound the standard solutions used in calibrating the instrument in such a manner that these standards contain quantities of the interfering molecules in proportions similar to those quantities contained in the solutions to be analyzed. This procedure has been adopted, and in applications where one can accurately predict the composition of the solutions submitted for analysis, it has been satisfactory.

It is apparent, however, that such a procedure is not altogether convenient, since it requires the compounding of a series of standard solutions for each type of unknown which the laboratory must analyze. In other cases, where the chemical composition of unknowns may vary considerably from one sample to the next, the procedure is not feasible. For these reasons, a method was sought which would eliminate as nearly as possible the effect of foreign molecules upon the quantitative determination of the alkali metals.

In the usual spectrographic method of analysis it has been common practice to employ what is termed an "internal standard" (*3*) element to reduce the effect of variation of the light source, and other disturbing influences, upon the accuracy of the results obtained. The method consists of purposely adding to each sample to be analyzed a fixed quantity of some element (the internal standard) not normally occurring in the sample, before bringing the sample to excitation. Upon excitation, light is emitted by both the element being determined and the internal standard, and the ratio of the intensities of these two characteristic lights emitted is subsequently determined by photography and densitometry. The principle of the method is simply that any change in the source or any other factor influencing the light intensity emitted by one element similarly affects the internal standard element, so that the ratio of intensities obtained is constant regardless of the experimental conditions. Naturally it is advantageous to choose as an internal standard an element which bears excitation characteristics as similar as possible to the element being determined.

In attempting to apply the internal standard method to flame photometry, the choice of a suitable element as a standard was rather limited, because of the few elements excited at the flame temperatures used in the instrument. Since the flame photometer was primarily designed as an instrument for the determination of sodium and potassium, it was desirable, if possible, to choose one of the alkali metals as a standard rather than an alkaline earth metal. The spectra of rubidium, cesium, lithium, and indium were investigated as possibilities. For various reasons such as the intensity of light emitted by the element, the problem of filtering the characteristic radiation emitted, and the wavelength sensitivity of the photodetecting device to be employed,

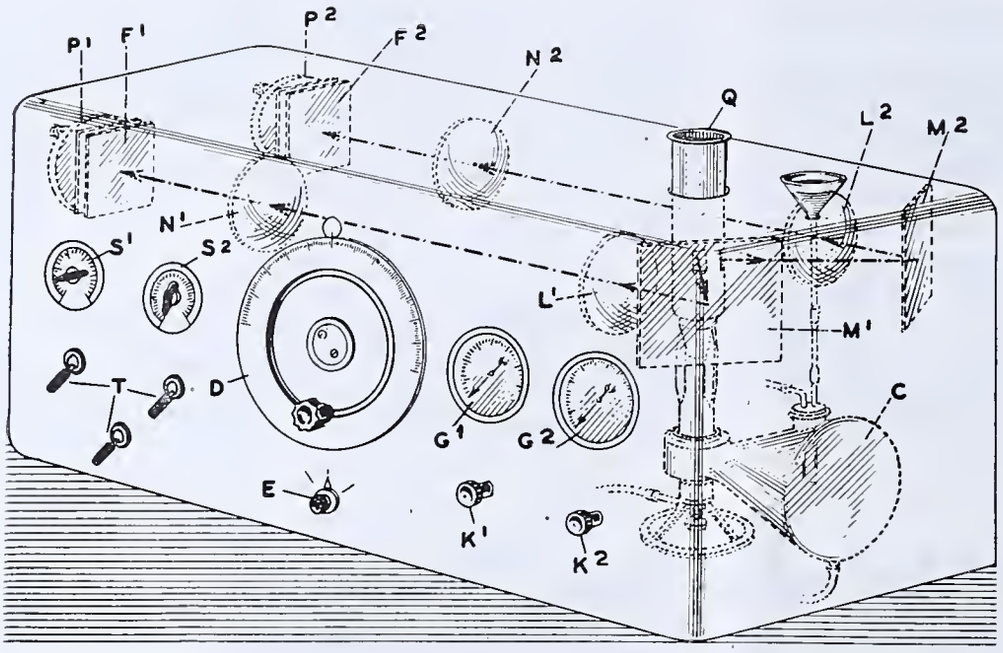

Figure 1. Internal Standard Flame Photometer

Q, chimney. M^1, M^2, mirrors. L^1, L^2, N^1, N^2, Fresnel lenses. F^1, sodium or potassium filters. F^2, lithium filters. P^1, P^2, barrier layer photocells. *C*, atomizing chamber. G^1, G^2, gas and air pressure gages. K^1, K^2, gas and air regulator knobs. *D*, main potentiometer dial. *E*, compensating rheostat. *T*, toggle switches. S^1, S^2, sensitivity adjustment, coarse and fine

all except lithium were rejected. The only serious objection to the use of lithium is the fact that the element is relatively abundant and may therefore occur as an impurity in certain types of samples, especially those of mineral origin. The light emitted by lithium is sufficiently intense, and the only line emitted (6708 Å.) is favorably placed about equidistant in the spectrum between the lines of sodium and of potassium. The problem of filtering the three radiations sufficiently free from one another was accomplished with Corning glass filters and a special liquid filter developed in these laboratories (*4*) which consists of cupric chloride dissolved in concentrated hydrochloric acid.

INTERNAL STANDARD FLAME PHOTOMETER

To test the internal standard method as applied to flame photometry, it was necessary to construct a special instrument housing two light paths and two photocells. The instrument built (Figure 1) was similar in general design to the flame photometer previously described (*1*).

Light leaves the flame through two rectangular apertures on opposite sides of the chimney, *Q*, each beam being reflected into an optical system similar to that previously used. The lens, L^1 or L^2, nearest the chimney casts an enlarged image of the aperture on the second lens, N^1 or N^2, filling this lens as nearly as possible with the rectangular image produced. The second lens casts a slightly reduced image of the first lens upon a round photocell, P^1 or P^2, just filling the active area. This system provides high light-gathering power yet allows the photocells to be well removed from the heat of the source. The barrier layer photocell has been retained as the light-detecting device. Before one photocell is placed the set of lithium filters, while sodium or potassium filters may be interchanged before the other photocell. [If desired, separate light paths and photocells may be provided for the sodium and potassium (making a total of three optical systems) and the desired sodium or potassium cell switched into the circuit electrically.]

Gas and air supplied to the burner and atomizer, respectively, are regulated and measured by appropriate regulators and gages, G^1 and G^2. The stainless steel hypodermic needle atomizing unit, and the conical-shaped atomizing chamber, *C*, used in the previous instrument were left unchanged.

The circuit used for measuring the ratio of the light intensities between the internal standard and the element being determined is shown in Figure 2. (Actually the circuit employed is not a true ratio-measuring device but rather a compensated circuit. At balance, however, the potentiometer reading obtained is very nearly proportional to the ratio of the light intensities on the two photocells.) Several of the "bucking circuit" arrangements suggested in the literature (*2*) were tried but none proved satisfactory at the low light levels encountered in the photometer. The circuit used maintains a resistance of some 10,000 ohms across each photocell, which seems about proper for the damping characteristics of the galvanometer. The 10,000-ohm potentiometer, *D*, was coupled to a 20-cm. (8-inch) circular dial on the front panel, making possible the reading of the potentiometer to within ±0.2% of full scale. A small 500-ohm rheostat, *E*, was added to one end of the slide wire for slight compensating adjustments found necessary from time to time during operation. The box-type galvanometer used in the previous instrument (G.E. 32C-245-G9) made a satisfactory null indicator for the instrument. As formerly, the galvanometer was connected to the photometer by jacks.

Also shown in Figure 2 is the circuit used in the previous method of flame photometry. Although not shown, the instrument was so arranged that either circuit shown in Figure 2 could be utilized by merely throwing toggle switches, *T*. The instrument could thus be used as previously described—to measure the direct or absolute intensity of the sodium or potassium wave lengths, now termed the "absolute method" of flame photometry, or the ratio of light intensities of sodium versus lithium or potassium versus lithium, termed the "internal standard method" of flame photometry. This arrangement was very convenient in making comparisons of the two methods.

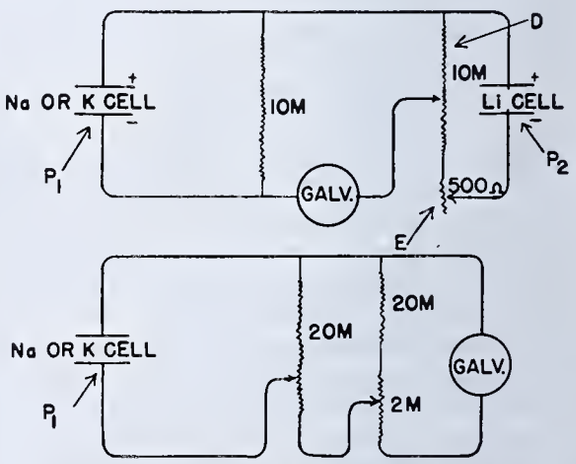

Figure 2. Circuits of Internal Standard Flame Photometer

Ratio circuit above, absolute circuit below

ANALYTICAL PROCEDURE

The procedure employed in making use of the internal standard method is similar in principle to that employed in the absolute method, in that the instrument is first calibrated with solutions of known concentration. All the standards, however, are prepared to contain a fixed amount of a soluble lithium salt. Lithium sulfate has generally been employed for this purpose, as the salt is rather easily prepared in a form relatively free of sodium and potassium.

Reagent quality lithium sulfate is precipitated as the fluoride by adding ammonium fluoride in slight excess. The lithium fluoride is then washed with cold distilled water, dried, and con-

verted back to the sulfate in platinum ware by the addition of concentrated sulfuric acid and heating. Since the sodium and potassium fluorides are several times more soluble than the lithium salt, the procedure appears satisfactory.

In practice, all sodium standards were made to contain 1000 p.p.m. of lithium, and amounts of sodium ranging from 0 to 90 p.p.m. (It was found by experiment that this quantity of lithium gives an electrical response equal to the response of 95 p.p.m. of sodium with the particular filters and photocells employed in the instrument.) The standards thus prepared are successively introduced into the instrument and in each case the potentiometer is so adjusted as to cause no current to flow through the galvanometer. A potentiometer dial reading is taken for each standard and a calibration curve is prepared. The curve obtained is smooth, and fairly linear (Figure 3).

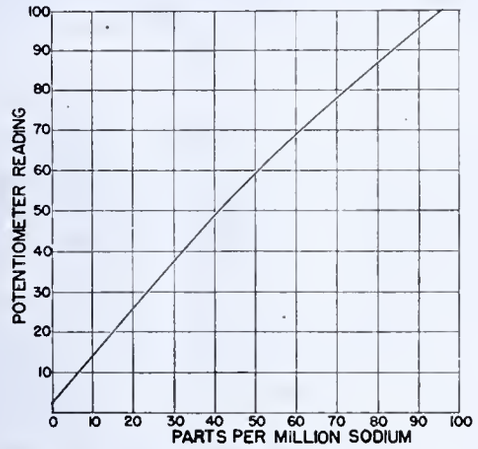

Figure 3. Typical Calibration Curve for Sodium as Obtained by the Internal Standard Flame Photometer

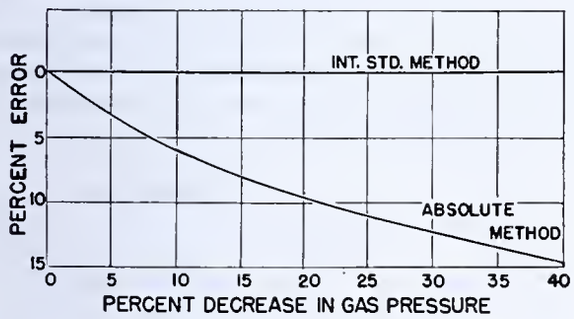

Figure 4. Effect of Variation of Gas Pressure

To determine the sodium content of a sample, an aqueous solution is appropriately pipetted into a volumetric flask, so that upon dilution the sodium content will fall below 90 p.p.m. A sufficient quantity of a stock lithium sulfate solution is then added to bring the lithium concentration to 1000 p.p.m. upon dilution. The sample thus prepared when introduced into the instrument yields a potentiometer reading which is readily converted into parts per million of sodium by use of the calibration curve.

In actual operation the calibration curve remains well fixed after establishment. The standard solution containing 0 p.p.m. of sodium and 1000 p.p.m. of lithium will read near the lower end of the main potentiometer scale and the 90 p.p.m. of sodium standard will read near the upper end of the scale. When employing the flame photometer for analysis it will be found wise to check the upper and lower ends of the scale occasionally with the standard solutions. Unless the flame in some manner becomes contaminated with sodium (dust in the atmosphere, etc.), the lower end of the calibration curve will remain well anchored. If drift occurs at the upper end of the scale, compensation may be made by adjustment of the secondary slide wire, *E*, to restore the original reading of the standard.

A similar procedure is used in the determination of potassium, with the exception that the solutions are prepared to contain 200 p.p.m. of lithium. This amount of lithium balances about 95 p.p.m. of potassium. The lithium concentrations chosen were such as to balance convenient working concentrations of sodium and potassium, but more or less lithium may be added to prepare a series of standards covering greater or lesser ranges of concentration of sodium and of potassium.

The amount of sample required for a single sodium or potassium determination is about the same as that required by the previous instrument (*1*). Samples of as little as 3 ml. of solution have been analyzed, but a larger quantity of sample (say 10 ml.) is preferred. Although the authors prefer working in a concentration range of 1 to 100 p.p.m. of sodium or potassium, concentrations as low as 0.1 p.p.m. of sodium or 0.5 p.p.m. of potassium have been determined. In working at concentrations below 5 p.p.m. of sodium or potassium a special set of standard solutions containing less lithium should be used for calibration of the instrument.

ACCURACY

In order to determine the accuracy of the internal standard method of flame photometry, a series of 100 solutions containing known concentrations of sodium was prepared and analyzed by the method. A similar series was studied in the case of potassium. These solutions were prepared from C.P. sodium and potassium chlorides and lithium sulfate prepared as previously described. The average error of a single determination of sodium was $\pm 1.24\%$, while for potassium a figure of $\pm 1.01\%$ was obtained. The sign of the error was found to be random. These figures show conclusively the superiority of the internal standard method over the absolute, for in a similar experiment using the absolute method, the average error of a single determination was shown to be $\pm 3\%$ of the amount of element present.

Further experiments have been conducted which show the superiority of the internal standard method with respect to common interferences which may beset the absolute method. These detrimental effects may be divided into at least four classes: effect of variation of gas pressure, effect of variation of air pressure, effect of foreign molecules and ions, and effect of viscosity of the sample.

In order to determine the magnitude of these various effects, standard solutions were prepared and analyzed by both the internal standard and the absolute method. These solutions all contained 50 p.p.m. of sodium (as the chloride) plus varying amounts of the interfering substances (except in the case of the effect of sodium chloride, in which case 50 p.p.m. of potassium was the concentration chosen). The standards used in the evaluation of the internal standard method contained, in addition, 1000 p.p.m. of lithium.

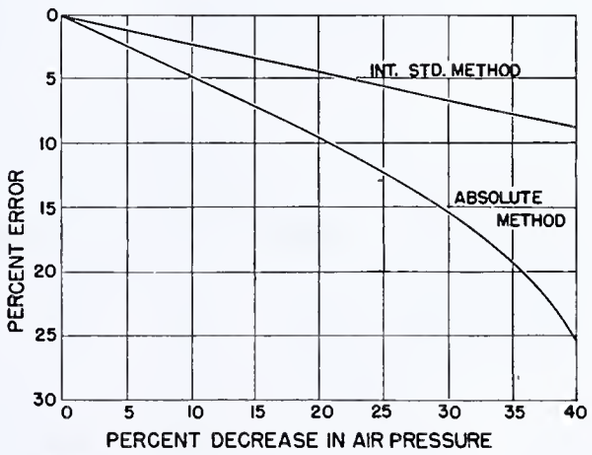

Figure 5. Effect of Variation of Air Pressure

In a separate series of experiments, it was subsequently shown that the percentage error produced by a given quantity of interfering ion or molecule is independent of the amount of sodium or potassium present in the sample over a concentration range of 10 to 100 p.p.m.

INTERFERENCES

GAS PRESSURE. The effect of lowering the gas pressure from that normally used in the operation of the flame photometer [0.21

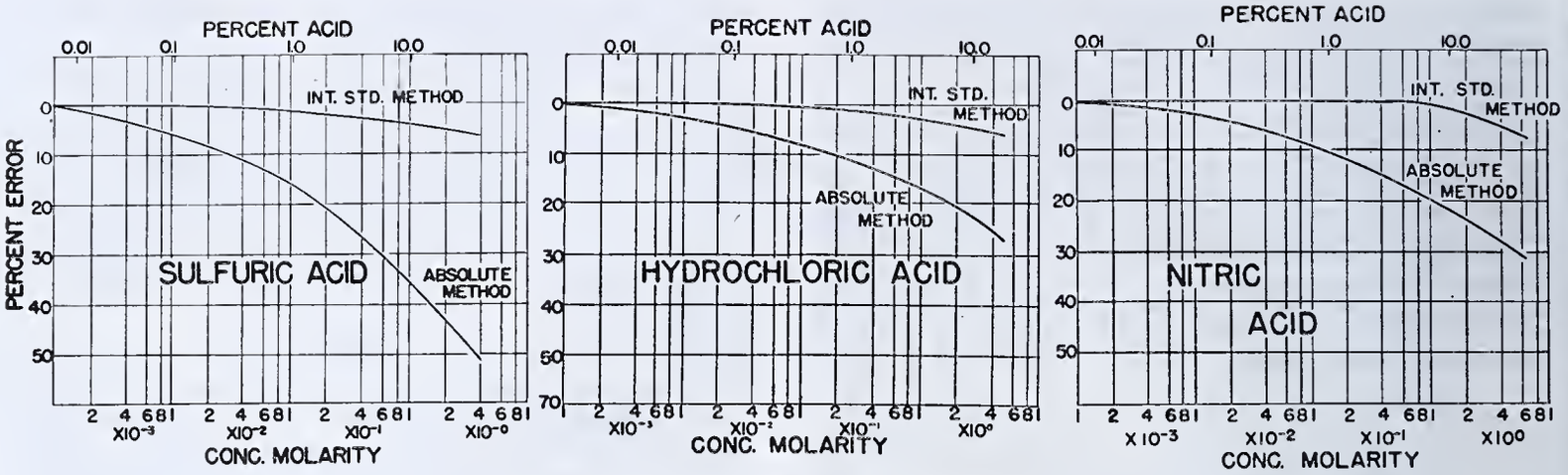

Figure 6. Effects Produced by Typical Acids

kg. per sq. cm. (3 pounds per sq. inch) of propane] is shown graphically in Figure 4. It is immediately noted that the gas pressure may be lowered by 33% [from 0.21 kg. per sq. cm. (3 pounds) down to 0.14 kg. per sq. cm. (2 pounds)] using the internal standard method with no appreciable change in the quantity of metal determined in a sample, while a similar change of gas pressure lowers the result obtained in using the absolute method by some 12.5%. The obvious superiority of the internal standard method should now make feasible the operation of flame photometers directly from city gas mains where variations in gas pressure previously made such operation very questionable.

Air Pressure. That variations of flame photometer readings are much more dependent upon air pressure fluctuations than upon gas pressure changes is immediately apparent from a study of Figure 5. The internal standard method does not completely eliminate the effect of variations in air pressure at any range. The magnitude of the effect, however, is reduced by a factor of about 2.5. Thus, a reduction of air pressure from the standard operating pressure of 1.05 to 0.84 kg. per sq. cm. (15 to 12 pounds per sq. inch) lowers the absolute reading obtained by some 10% while reducing the internal standard result by only about 3.5%. Again the internal standard method of photometry is distinctly superior to the absolute, but adequate pressure regulation will remain necessary for the best results.

Foreign Molecules. Shown in Figures 6, 7, and 8 are plots of the errors resulting in flame photometry which occur when certain inorganic acids and salts, as well as organic molecules, are introduced into the solutions being analyzed for sodium and potassium. In every case shown, where the absolute method of analysis is employed, the introduction of acids or salts into the solution reduces the light emitted by the sodium or potassium. If a sufficient quantity of the interfering substance is added, the internal standard method also will usually produce a low result, but not until considerably higher concentrations of the interfering molecules have been added do these effects begin to appear. In the case of sulfuric acid, for example, the introduction of 0.2 mole of acid per liter (2% acid approximately) results in an absolute reading for sodium which is some 21% lower than the true

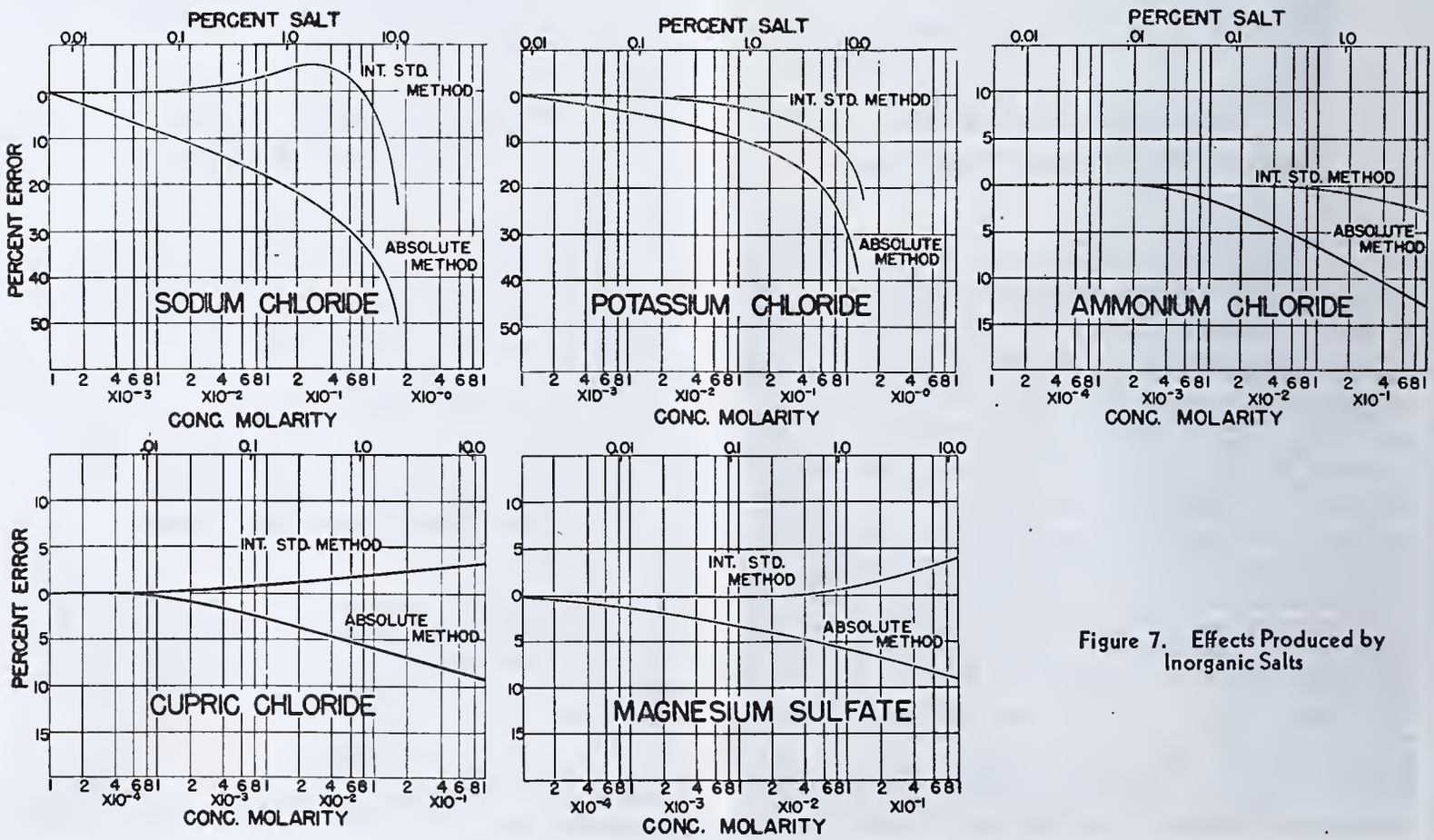

Figure 7. Effects Produced by Inorganic Salts

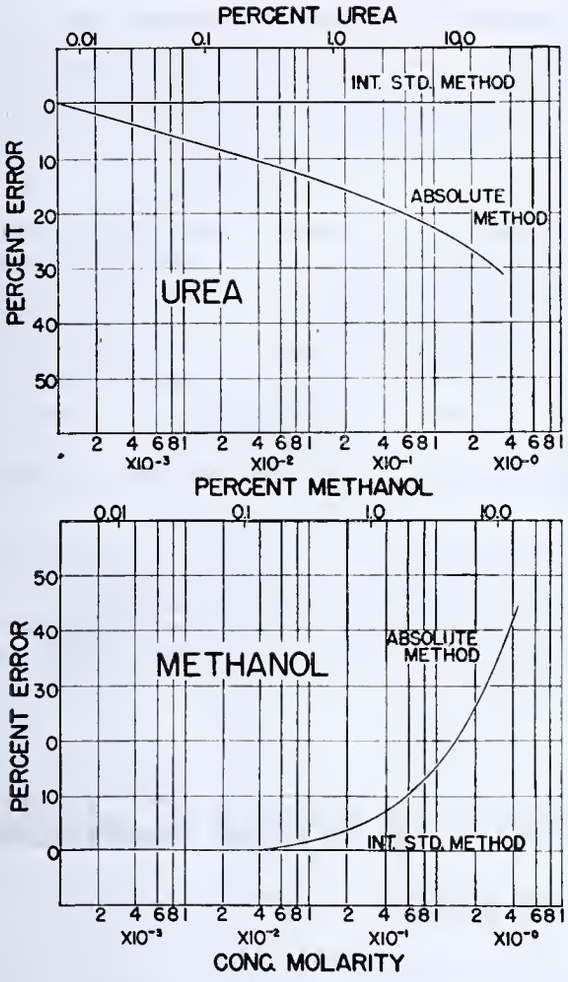

Figure 8. Effect Produced by Organic Molecules

value, while by the internal standard method the result obtained is but 1.5% lower than normal. Hydrochloric and nitric acids behave similarly, severely depressing the absolute readings obtained while not affecting the internal standard method until relatively high concentrations of these acids are present in the sample. Other acids such as phosphoric, hydriodic, and hydrobromic have been found to behave similarly. It may be noted from the graphs that from 50 to 200 times the amount of acid may be tolerated by the internal standard method as by the absolute—i.e., before an equal suppression of the result is noted.

Salts of most types depress the readings obtained by the absolute method of flame photometry much as do the acids. The effects of five different salts are shown in the graphs—namely, ammonium chloride, sodium chloride, potassium chloride, magnesium sulfate, and cupric chloride. These salts begin to depress the absolute readings when present at approximately the same range of concentration as do the acids. It is obvious, therefore, that the neutralization of an acid in a sample with, for example, ammonium hydroxide, will not lessen the ill effect of the acid.

Potassium chloride affects the determination of sodium much as does ammonium chloride. Similarly, sodium chloride will depress the readings obtained in a potassium determination if present in sufficient quantity. This effect should especially be remembered if any attempt is made to determine a trace of one of these elements when combined with large quantities of the other.

Of interest also are the effects produced by two of the salts, the magnesium sulfate and the copper chloride, which tend to raise the result obtained by the internal standard method rather than to produce a low answer as they do by the absolute method. Two explanations for this behavior are possible: either that the salts depress the light emitted by lithium to a greater extent than that by sodium, or that a certain small amount of light is emitted by copper and magnesium which will pass the sodium filter. The former reason may apply to the magnesium in the case of sodium determinations, but the latter reason may apply to the copper salt, as it is known that copper salts do emit a green band, some of which may pass through the sodium filters.

Figure 8 shows the effects produced by two common organic molecules. Urea behaves very similarly to the acids and salts—the effect quantitatively being of the same order of magnitude. Methanol, however, behaves in a manner entirely different from any substance previously mentioned in that it increases the light emitted by the flame. Ethanol has been observed to exhibit a similar behavior.

Viscosity. As might be expected, samples which contain sodium or potassium and some additional agent which considerably increases the viscosity of the sample tend to read lower than solutions of sodium or potassium of normal viscosity—when the determination is made by the absolute method. The explanation is obvious in that the absolute method depends upon a constant rate of atomization in the standards and in the unknowns. Higher viscosities decrease the rate of atomization of the sample, so that fewer atoms are introduced into the flame in a given time, and consequently less light is emitted by the flame.

Figure 9 shows the comparative effects of viscosity upon the internal standard and absolute methods. Sucrose was added to the samples to increase the viscosity. In an extreme case where 40% sucrose was added to the sample (viscosity 5.1 centipoises) the absolute method produced a result 42% below the true value, while the result obtained by the internal standard method was but 11% low.

A study of the flow rates of the solutions through the atomizer shows that the light emitted is decreased by a lesser amount than would be expected from the decrease in the rate of atomization. Undoubtedly the presence of the sucrose gives rise to some type of interference, apparently behaving as does methanol—that is, to produce more light.

Surface Tension. It was thought probable that variations in the surface tension of solutions might give rise to some change of the atomization characteristics of solutions being atomized into the flame photometer and hence lead to an increase or decrease in the light emitted by the flame. The effect of surface tension was studied by adding the ammonium salt of Aerosol OT (a product of the American Cyanamid Company; the ammonium salt was specially prepared in these laboratories) to solutions of sodium and potassium chlorides. Using the absolute method, no effect was observed upon the light emitted by the flame although the surface tension varied from that of water (ca. 72 dynes per centimeter) down to 28 dynes per centimeter. An explanation might lie in the known fact that a time factor is involved in lowering of the surface tension of freshly formed surfaces when surface-active agents are used to lower the tension. If this factor does explain the failure of surface-active agents to alter the atomization characteristics of a solution, there then remains a

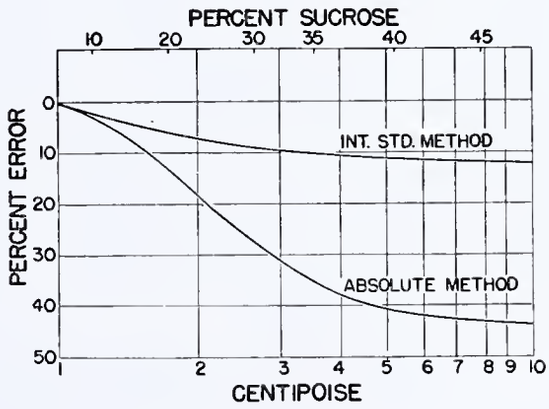

Figure 9. Effect of Viscosity

question as to whether the surface tension of a solution introduces an effect upon the light emitted by a flame. For example, the increased amount of light emitted by a solution containing alcohol may be partly due to the altered surface tension of the solution. At the present time, no attempt has been made to study this effect further.

PROPOSED ALTERNATIVE PROCEDURE

Certain analysts in considering the internal standard type of flame photometer may propose that the previously described type of flame photometer, employing a single photocell, be used in a quasi-internal standard method by adding lithium to each sample and measuring individually the intensity of the lithium and sodium lines. This, of course, could be readily accomplished if a suitable method of mechanically interchanging the filters before the photocell were devised.

Such a procedure might serve well in eliminating the effects of foreign ions and molecules upon the flame, but would not eliminate the effects of variations in gas, air pressure, or other momentary changes which constantly alter the intensity of the flame. The error introduced by variation of the flame could, of course, be substantially reduced by alternately measuring the sodium and lithium intensities several times on each sample and obtaining an average ratio value.

Although such a procedure may be fairly satisfactory, the time involved for each analysis would be considerably increased, and in general the method would lack elegance.

CONCLUSIONS

The advantages of making use of an internal standard in flame photometry are considerable. In most analytical work the additional time and energy required in using the method are small because of the usual necessity of diluting the sample before analysis and the addition of the internal standard during this step is simple. However, judgment must be applied in making use of the flame photometer in analyzing new types of materials, and therefore a certain amount of careful investigative work should be done before attempting any previously untried determination.

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Determination of Nitrogen in Refractory Metal Carbides and Their Compositions

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A procedure is described for determining nitrogen in refractory metal carbides by the solution and distillation method. No special apparatus is required and very precise results are obtained. The nitrogen contents of several representative carbides and compositions are given.

THE great increase in the past few years in the use of cemented compositions of the refractory metal carbides for cutting tools, dies, wear parts, and other applications has accelerated metallurgical investigation of these materials. Since several of the elements used in these compositions readily form nitrides when they are present in the environment in which the carburization takes place and since there is also a possibility of nitride formation during sintering operations in some commercially used atmospheres, investigation of nitrogen content of these materials seemed of interest. It was first necessary to develop a procedure for this work and the results of this development and the method as finally used are reported herewith.

Although a great deal has been published in the literature on the determination of nitrogen in steel, the authors found only one reference to its determination in carbides (*3*). This paper incidentally contains a very complete and worth-while bibliography on the determination of nitrogen in iron and steel. Phragmen and Treje (*3*) investigated both the vacuum fusion and solution and distillation methods. In the case of the solution and distillation method they used a refluxing technique for solution with special apparatus and sulfuric acid plus potassium bisulfate as the solution agents. For titanium carbide their results show a much lower recovery with the solution and distillation method than for the vacuum fusion procedure using sodium peroxide. Hence, when applied to titanium carbide, which is a constituent of many commercial carbide compositions and is a very likely source of nitrogen, the solution and distillation procedure of Phragmen and Treje appears unsatisfactory.

It was the authors' desire to develop a solution and distillation method without the use of special apparatus, but it is obvious that the problem would be one of developing a method of obtaining complete solution. These carbides and their compositions are very refractory and are ordinarily insoluble without resort to oxidizing reagents or fusion. As shown in Table I, several unsatisfactory procedures and modifications were investigated.

Table I. Procedures Investigated

	Solution Method	Results
1.	Fuming sulfuric acid plus sodium sulfate (*2*)	Partial solution and low results
2.	Fuming sulfuric acid plus sodium thiosulfate	Usually incomplete solution. Erratic results
3.	Fusion with potassium bisulfate and solution of melt in hydrochloric acid	Results low when fusion was complete
4.	Fuming with sulfuric acid plus potassium bisulfate plus hydrofluoric acid	Very destructive to glassware
5.	Heating in a stream of hydrogen with copper oxide and lead oxide catalysts and absorption of ammonia in sulfuric acid	Results very low

The use of perchloric acid as recommended in some procedures for determining nitrogen in alloy steels was not tried, inasmuch as there has always been a question of the loss of nitrogen by oxidation in even short periods of fuming. Since extended periods were necessary for these materials, the loss of nitrogen seemed certain.

It was then found that by a careful technique, extended fuming with a mixture of sulfuric acid, potassium bisulfate, and either cuprous oxide or selenium oxychloride could be carried out and complete solution could be obtained. In some instances this required as much as 184 hours for the solution of a 1-gram sample but there was no evidence of loss of ammonia. This procedure was adopted as described in detail in this paper.

APPARATUS

The distillation apparatus used is shown in Figure 1. This apparatus is a combination of that described by Lundell, Hoffman, and Bright (*2*), and one developed a number of years ago at Battelle Memorial Institute. The large tube leading from the funnel to the bottom of the distillation flask facilitates the introduction of the sample solution, which is particularly necessary with solutions of the carbides since they often contain insoluble matter. The small capillary tube extending near the bottom of the flask permits the use of a small stream of carbon dioxide–free air (obtained by passing the air through a tower filled with carbon dioxide absorbent) to ensure steady distillation and freedom from bumping. The construction of the trap is particularly effective in preventing carry-over of any of the strong sodium hydroxide solution.

SOLUTIONS REQUIRED

Ammonia-Free Water prepared over Devarda's alloy (*4*).

Selenium Oxychloride Solution, 1.2% in concentrated sulfuric acid, specific gravity 1.84.

Tartaric Acid, 50%, prepared with ammonia-free water.

Sodium Hydroxide Solution, 60%. Dissolve 600 grams of the reagent in 1000 ml. of distilled water and digest overnight on the water bath with a zinc-copper couple.

Sodium Hydroxide, 0.01 *N*. Standardize against Bureau of Standards acid potassium phthalate, No. 84.

Sulfuric Acid, 0.01 *N*. Standardize against 0.01 *N* sodium hydroxide.

Methyl Red Indicator Solution, 0.1% in 95% ethyl alcohol.

For higher nitrogen contents 0.1 *N* sodium hydroxide and sulfuric acid prepared in the same manner are used.

PROCEDURE

The material to be analyzed should be as finely divided as possible, and in any case under 200 mesh. This may be readily accomplished by grinding the material in a motor-driven mortar and pestle fitted with cemented carbide insert and tip, respectively. The composition used should be of the straight tungsten carbide type to minimize contamination.

Transfer a carefully weighed 1.0-gram sample to a dry 250 ml. Erlenmeyer flask and add 15 grams of potassium bisulfate, reagent grade, 30 ml. of sulfuric acid, and 1 ml. of selenium oxychloride solution. Place the flask on a hot plate regulated at a temperature to keep the solution just fuming, and invert a small beaker over the neck of the flask. In this way fuming can be continued for many hours without replenishing the acid. Continue the heating until solution is complete and cool to room temperature. Any undissolved residue may be readily detected by observing whether black residue remains when the flask is swirled. Carefully add 50 ml. of 50% tartaric acid solution and then 75 ml. of ammonia-free water. Boil gently for 5 minutes or until all soluble salts are dissolved.

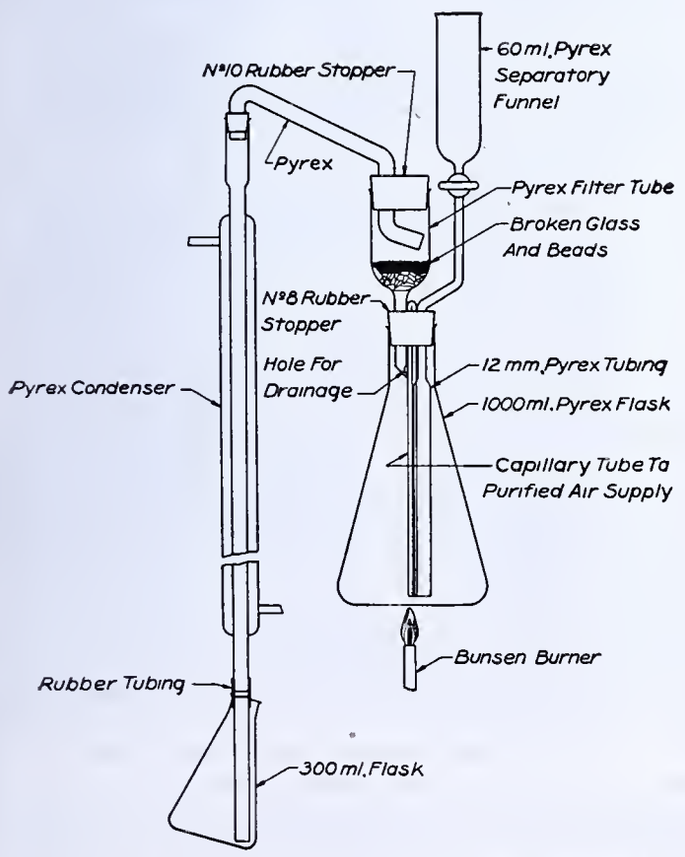

Figure 1. Nitrogen Distillation Apparatus

Table II. Typical Results

Material	General Composition	N_2 Found, %
Tungsten carbide + ammonium sulfate solution[a]		2.056
Titanium carbide		0.982
		0.978
Tungsten carbide 1		0.127
		0.113
		0.119
Tungsten carbide 2		0.122
		0.119
		0.122
		0.121
		0.121
Titanium nitride		16.24
		16.19
Cemented carbide composition	W + Ti + Cb + Ta	0.0252
		0.0252
	W + Ti + Cb + Ta	0.0258
		0.0258
	W + high Ti	0.35
	W + low Ti	0.11
		0.11

[a] Equivalent to 2.067% nitrogen.

When solution is nearly complete, prepare the distillation apparatus. Rinse out the entire distillation flask assembly with ordinary distilled water and measure 150 ml. of 60% sodium hydroxide solution into the flask. Add 400 ml. of distilled water and assemble the apparatus. Place a 300-ml. Erlenmeyer flask under the condenser outlet tube and start a gentle stream of air through the apparatus. Then apply the heat and continue to heat at as rapid a rate as is consistent with the operation of the trap until 150 to 200 ml. of distillate have been collected. Remove the flask and discard. Rinse the outlet tube down with ammonia-free water, place under the outlet tube a flask, rinsed with ammonia-free water, to which a few drops of methyl red indicator solution have been added, and distill over 50 ml. If the indicator remains pink the system is free of ammonia and distillation of the sample may proceed.

Remove the heat from the distillation flask, remove the collecting flask, and substitute another rinsed flask into which 5 ml. of 0.01 *N* sulfuric acid (or 0.1 *N*) have been pipetted. Also add 4 drops of methyl red indicator solution to the flask. Increase the air flow slightly and transfer the solution of the sample to the funnel. Slowly open the funnel stopcock and allow the solution to flow into the flask at such a rate that the reaction is not too violent with resultant splashing of the solution into the trap. Some dilution of the sample solution may be desirable. When the solution is all transferred, rinse the sample flask and funnel three times with ammonia-free water, finally adding a total of 500 ml. Replace the flame, slow the air stream somewhat, and proceed with the distillation. Observe the receiver for any evidence of neutralization of the acid and add more by pipet as needed to keep the indicator pink. When 200 ml. of distillate have been collected, remove the flask from the outlet tube while rinsing it down with ammonia-free water.

Titrate the excess sulfuric acid with 0.01 *N* sodium hydroxide (or 0.1 *N*) until the pink color just disappears. Add 2 more drops of methyl red indicator solution and continue titrating to a full yellow color.

Carry a blank using the same volume as in a sample run through all steps of the operation except the fuming.

On a 1-gram sample, 1 ml. of 0.01 *N* sulfuric acid used to absorb ammonia is equivalent to 0.0140% nitrogen in the sample.

RESULTS

Typical results obtained (Table II) show clearly that the reproducibility is excellent and that added known amounts of ammonia are recovered. Unfortunately, there are as yet no standards available for this class of materials, so that the procedure could not be tested in this manner.

In lieu of the use of standards, some confirmatory results are included. Tungsten carbide is shown not to contain much nitride and this confirms the statement made by Kieffer and Hotop (*1*) that tungsten does not form nitrides below 2300° C. Tantalum and columbium might be expected to behave similarly.

Hence, titanium is the greatest source of nitrogen in these compositions and the results show that titanium nitride is decomposed, so that even were the sample not completely decomposed, the nitride would be recovered.

ACKNOWLEDGMENT

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Evaluation of Dispersions by a Novel Rheological Method

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A technique for the rapid evaluation of dispersions and dispersing agents is presented. The test comprises mixing the dry powder with the dispersing solution, measuring the amount of liquid required to produce two characteristic and reproducible consistencies, and observing the rheological phenomena of the mixture. A good dispersion displays active dilatancy at high powder concentrations. A poor dispersion or flocculate is plastic over a wide range of concentrations and never displays any flow tendencies. A fair dispersion displays passive dilatancy at high concentrations and visible thixotropy at lower powder concentrations.

FINELY subdivided insoluble solids such as pigments display widely different characteristics when mixed with relatively small amounts of liquid. The fact that it requires more liquid to moisten some pigments than to moisten others is the basis of the oil absorption test (*6*) commonly used in the paint and allied industries. It is likewise known that the consistency of a given powder-liquid system can be changed by dispersing (deflocculating) the clusters of powder particles present in the mixture. Deflocculation is accompanied by such a sharp reduction in yield value that a stiff paste becomes fluid (*7*). Consequently a paste of higher powder concentration can be produced in a dispersed than in a flocculated system.

As used in this paper the term "dispersion" denotes a powder-liquid system in which the primary particles are largely independent of each other. Used in this manner its meaning is not identical with the generic term, "dispersion", often applied to any two-phase system, flocculated or otherwise. A "flocculate" denotes a powder-liquid system in which the primary powder particles are held together in clusters by adhesion tension (*1*, *8*). Clear distinction is made in this paper between "dispersing agents" which serve the function of deflocculating or dispersing flocculated systems, and "wetting agents" whose function it is to aid in wetting water-repellent particles by a mechanism of lowering surface and interfacial tension.

The authors have studied more closely the changes in consistency brought about by variations in the state of flocculation or deflocculation (dispersion) of a concentrated powder-liquid system. Like Fischer and Jerome (*3*), they find that qualitative and quantitative differences in the rheological behavior (consistency) of the mixture are so pronounced and typical that these characteristics, in turn, can be used as a measure of dispersion.

Based on this observation, a simple and comprehensive technique for evaluating dispersions and dispersing agents has been developed. Such a method should be welcome to those active in the field of dispersion where, at present, guesswork in evaluating dispersions is often used for want of a simple and quick testing method.

SCOPE

The method of testing for dispersion here presented applies to dry, insoluble powders incorporated into a continuous liquid phase. (Powders which swell in the liquid medium, such as clays in water, can be tested only after they reach their swelling equilibrium. The results must be interpreted with caution.) The test comprises mixing the powder with the dispersing solution, measuring the amount of liquid required to produce two characteristic and reproducible consistencies, the "wet point" and the "flow point", and observing the attendant rheological properties of the mixture. Thus far, the test has been used chiefly for aqueous systems, and the present paper deals with this method of testing for dispersion only as it applies to aqueous systems. However, with but minor modifications, the test can be used for nonaqueous systems as well. In some of its aspects it is an art more than a scientific method. Its chief limitation is that it tests dispersions at high concentration, and the results cannot always be applied to more dilute dispersions. However, discrepancies between the degree of deflocculation of such mixtures at high and at low concentration seem to be the exception rather than the rule.

RHEOLOGICAL DESCRIPTION

The rheological behavior of concentrated powder-liquid systems falls into three distinct, recognizable groups, depending on whether the powder is flocculated, well dispersed, or only partly dispersed.

FLOCCULATES. As a liquid is gradually combined with a powder, the mixture becomes dull in appearance and puttylike in consistency (see Figure 1, upper photo). As more liquid is added, the system remains dull and displays adhesive qualities which cause sharp peaks to appear during kneading. With continued additions of liquid, the mixture becomes proportionately softer, but remains dull in appearance even upon tapping or patting. Finally, as still more liquid is added, the mixture reaches a stage where, though pastelike, it falls or drops under its own weight from a vertically held spatula. This plastic flow behavior with a pronounced yield value over a wide range of concentrations is characteristic of flocculates or poor dispersions.

GOOD DISPERSIONS. As the liquid is combined with the powder, the mixture displays great resistance to sudden pressure or to kneading, turning dull and ridged in appearance. Yet a few moments afterwards the same material coalesces, becomes glossy and smooth, and flows of its own accord if left undisturbed (see Figure 1, center photo). Even when the flow—from a spatula, for instance—is slow, owing to a very high concentration of powder, the falling material spreads out on a glass plate in a glossy pool. At slightly lower concentrations of powder, the material becomes much more fluid, running off in long, fine strings from a horizontal spatula, yet offers a high resistance to sudden thrusts with a knife. This phenomenon is called "active dilatancy" (*4*) and it is indicative of a high degree of dispersion (*2*, *5*). Active dilatancy is observed in powder-liquid systems of high powder concentration only. A small additional amount of liquid will turn such a mixture into a mobile fluid. In the dilatancy range, the

quality of the dispersion can be judged by the degree of spontaneous flow. The longer and finer the honeylike strings of the mixture, with a given powder at a given concentration, the better the dispersion.

Fair Dispersions. As the liquid is gradually combined with the powder, the resultant mixture appears dull, as is likewise true in a flocculate. Yet when gently tapped, the material turns glossy and begins to flow, but ceases its flow as soon as the disturbance is discontinued (see Figure 1, lower photo). This phenomenon is called "passive dilatancy" (*4*). When more liquid is added, the material loses its resistance, and when agitated, seems mobile. Yet when left undisturbed, it sets—i.e., it is visibly thixotropic (*9*) and displays the reversible isothermal sol-gel transformation which characterizes all thixotropic systems. When dropped, it shows the rounded peaks and craters typical of such systems. Even when rather dilute, it will neither flow in strings nor spread out in a thin layer when poured onto a glass plate.

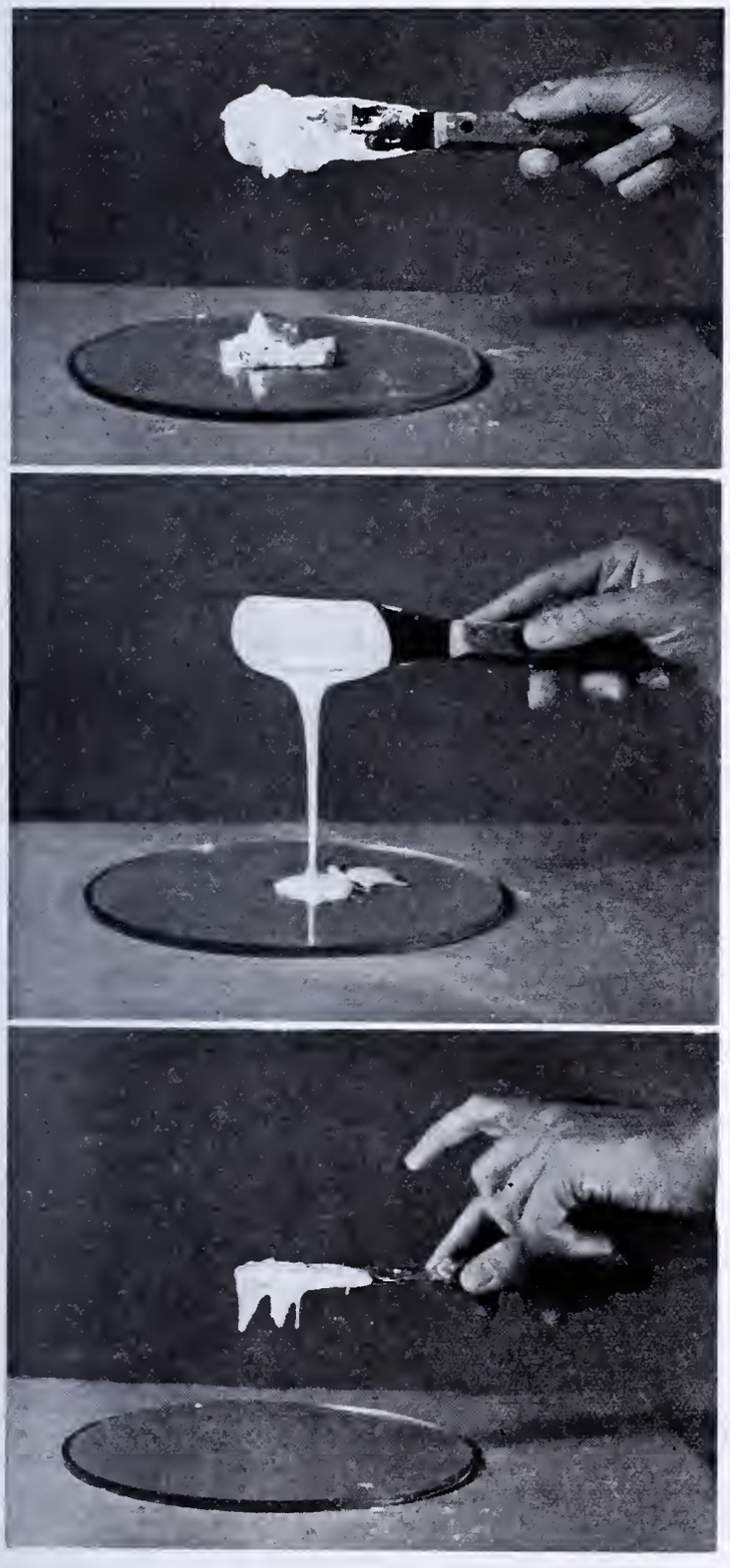

Figure 1. Powder-Liquid Dispersions

Upper. Mixture of 85% water and 15% powder by volume. Plastic, poor dispersion
Center. Mixture of 60% dispersing agent solution and 40% powder by volume. Actively dilatant, good dispersion
Lower. Mixture of 65% dispersing agent solution and 35% powder by volume. Passively dilatant, fair dispersion

RELATIONSHIP OF GOOD, FAIR, AND POOR DISPERSIONS

While concentrated powder-liquid systems show the three general types of rheological behavior outlined above, no sharp line of demarkation between these groups exists. In fact, even within the same powder-liquid system, there may be produced—with an electrolyte, for instance—a continuous change from good dispersion (complete deflocculation) on one end of the scale, to complete flocculation or poor dispersion on the other end of the scale. This transition is accompanied by a corresponding change in rheological properties, both dilatancy and thixotropy.

If thixotropy be defined in terms of time of solidification (*4*), a poorly dispersed, plastic suspension may be regarded as an extreme case of thixotropy—viz., one in which the time of solidification is infinitely short and therefore thixotropy cannot be measured directly. At the other end of the scale, a good dispersion may be regarded as a thixotropic system in which the time of solidification is too long to be measured. In intermediate states, however, the association of particles takes place in measurable time intervals—e.g., in minutes. In these cases the time of solidification becomes tangible and thixotropy clearly noticeable.

The time of solidification of a suspension depends not on the degree of deflocculation alone, but also on concentration. In a given system, the time of solidification at high powder concentration is always shorter than at lower concentrations. Hence the concentrated powder-liquid systems described above, which stop flowing when vibration ceases, at lower concentrations turn visibly thixotropic—i.e., they will require many seconds or some minutes to set up. Passive dilatancy is merely a case of a rapid sol-gel transformation superimposed on dilatant flow.

Passive dilatancy at high powder concentration and visible thixotropy at lower powder concentration are indicative of a state intermediate between flocculation and dispersion. The residual forces between particles are strong enough to set up a loose network of particles, yet minor external forces are sufficient to break down the structure and to permit the particles or small clusters of particles, at least temporarily, to be independent of each other. This state of conditional dispersion is designated here as "fair dispersion".

For practical purposes—for maintaining a stable suspension, for instance—a system displaying some thixotropy is often preferable to a good dispersion displaying no thixotropy because a slight gelling of the system prevents the settling and hard caking of the powder.

EXPERIMENTAL PROCEDURE

Different though their rheological properties be, two characteristic consistencies can be produced in any concentrated powder-liquid system. These consistencies, "wet point" and "flow point" are wholly arbitrary but are easily reproducible and can, therefore, be used conveniently as markers. The wet point is defined as that stage in the moistening of a powder where the minimum amount of liquid is used to form a coherent mass. [The wet point corresponds roughly to the end point of the oil absorption test which measures the amount of oil required by a specified amount of pigment to form a paste (*6*).] The flow point is defined as that stage in the moistening of a powder where the minimum amount of liquid is used which will cause all or a substantial part of the powder-liquid mixture to flow or fall repeatedly from the vertical blade of a horizontally held spatula. The test is based on determining the amount of liquid required at the wet point and at the

flow point and observing the rheological characteristics at and between the two points.

Equipment. A glass plate, preferably a thick, round plate about 20 cm. (8 inches) or more in diameter.

A stainless steel spatula, preferably one with a broad, short, not too flexible blade—e.g., 10 cm. (4 inches long) and 3.1 cm. (1.25 inches) wide.

A buret with subdivisions of 0.1 cc.

A balance with accuracy of 0.1 gram.

Wet Point Determination. Weigh out the powder to be dispersed. If it is bulky and light, use 10 grams; if it is dense and heavy, use 20 grams. Place the powder on a glass plate and make a crater in the center of the powder. From a buret add some dispersing solution to the powder, running it into the crater until a little pool forms. Cover the pool with dry powder from the periphery and wait a few seconds until the liquid has soaked in. Then start kneading vigorously with the spatula until as much powder as possible has been wet. Where a water-repellent powder—e.g., sulfur—is to be dispersed it may be advisable to use a wetting agent in conjunction with the dispersing agent, as would be necessary in actual production. If a large proportion of the powder still remains dry, add more solution from the buret in an amount estimated to wet nearly all the remaining dry powder.

When the point is reached where nearly all the powder is moistened, interrupt the kneading action and scrape the material together into one heap and try by patting, pressing, and troweling with the spatula to form a moist, coherent mass. As that point is approached, add the solution dropwise with intermittent kneading and patting, so as not to overshoot the point where just enough and no more liquid is present to form all the powder into a coherent mass. Since the amount of liquid required at this point, the wet point, varies widely with different powders, an inexperienced operator may, at first, overshoot it and have to repeat the test, but with a little experience he will learn to approach the wet point rapidly and then reach it with a few more drops.

Flow Point Determination. After recording the volume of liquid needed to produce the wet point, add more dispersing solution dropwise and continue the kneading throughout until the flow point is reached and the requisite amount of liquid likewise recorded.

For well-dispersed systems the flow point is defined as the stage where a substantial portion of the material first flows off a vertically held spatula blade without producing jagged flow edges. Zinc oxide or barium sulfate dispersed with dialyzed waste sulfite liquor, in aqueous systems, and titanium dioxide dispersed with a 5% solution of a short oil oxidizing type alkyd in xylene, in nonaqueous systems, are good examples of this type of system.

The most characteristic feature of good dispersions is that here the flow point lies very close to the wet point. The better the dispersion, the narrower the gap between the two points until in some cases the wet point and flow point become practically indistinguishable.

For poor dispersions or for out-and-out flocculated systems, the flow point is reached when the paste drops off the vertically held spatula blade. This falling or dropping off is clearly different from the flow of good dispersions. Sometimes the whole mass comes off at once, leaving the spatula bare. More often, an appreciable amount of the mixture remains on the spatula. An example of a flocculated system is a mixture of zinc oxide and water, or, in nonaqueous systems, titanium dioxide in xylene.

In poorly dispersed or flocculated systems, the gap between wet point and flow point is very wide—in fact, so wide that sometimes the test is discontinued before the flow point is reached.

For fair dispersions, the flow point is harder to define and less reproducible than for either well or poorly dispersed systems. The material never really flows spontaneously but drops. Yet just as it breaks away from the spatula, it shows an elongation along the edge as though it were going to flow. The phenomenon is hard to describe but is easily recognized after a little experience. Usually, only part of the dispersion on the spatula comes off, especially if not all the material has been scraped up at the same time. This is due to the thixotropic character mentioned above, which introduces a time factor and also makes the flow point vary somewhat with the kind of kneading and mixing action given to the material. Often, by the time the first part of the material has left the spatula, the rest has set up. An example of a fair dispersion is a mixture of a rutile type titanium dioxide in a solution of dialyzed waste sulfite liquor.

The gap between wet point and flow point in fair dispersions is wide enough to be measured easily—in fact, sometimes it is so wide that one may think the system is flocculated. To decide definitely whether it is dispersed or not, the tapping test mentioned below should be made in all cases where the addition of a small amount of liquid to the wet paste does not produce flow point consistency—i.e., in all except actively dilatant systems.

Tapping at Intermediate Stage. When about 10 to 20% more liquid than the amount required at the wet point has been added and the material does not flow but remains dull and merely becomes softer, some of it is placed at the tip of a horizontally held spatula. The horizontal blade is tapped repeatedly, gently at first and if this is not sufficient to cause the material to move, more vigorously, near the handle with the index finger of the other hand. If the mixture turns glossy and flows over the edges of the blade in bands or strings when so tapped and flow is arrested in midair when tapping ceases, the material is passively dilatant (see Figure 1, lower). It is partly dispersed, or at least it can easily be made to disperse by such means as dilution and agitation, even though for a limited period of time only. However, if the material stays on the spatula and rises on it in scroll-like form or otherwise tends to decrease its surface area in contact with the blade, it is flocculated or contains some flocculated material in appreciable amount.

CHARACTERISTICS OF POWDER-DISPERSING SOLUTION MIXTURES

Good Dispersions

1. *At the Wet Point*
   - *a.* Shine without tapping or upon light tapping
   - *b.* Are dry and hard to knead
2. *At the Intermediate Stage* (defined as that mixture which contains about 10 to 20% more liquid than the wet point and has not reached flow point)
   - *a.* Lack distinct intermediate stage
3. *At the Flow Point*
   - *a.* Flow without tapping
   - *b.* Offer resistance to sudden pressure
4. Have a very small gap between wet point and flow point

Fair Dispersions

1. *At the Wet Point*
   - *a.* Shine on sharp tapping
2. *At the Intermediate Stage*
   - *a.* Flow on tapping only
   - *b.* Show some resistance to sudden pressure occasionally
3. *At the Flow Point*
   - *a.* Fall with elongation at the breaking line
   - *b.* Show no resistance to sudden pressure
   - *c.* Show visible thixotropy

Poor Dispersions and Flocculates

1. *At the Wet Point*
   - *a.* Remain dull even on tapping
2. *At the Intermediate Stage*
   - *a.* Rise on tapping
   - *b.* Show no resistance to sudden pressure
   - *c.* Possess marked plasticity (high yield value)
3. *At the Flow Point*
   - *a.* Fall without elongation at breaking point
4. Have a very large gap between wet point and flow point

EVALUATION

With the above-described testing procedure all pertinent information as to the quality of the dispersion and the quality and quantity of the dispersing agent needed can be obtained.

As a first step, every new powder to be tested becomes subject to a preliminary test with pure water instead of dispersing solution. This blank test will establish:

1. Whether the powder wets easily or requires the use of a combination dispersing-wetting agent
2. Whether the powder is flocculated or, like starch, for instance, has self-dispersing qualities. In the latter case, dispersing agents can be expected to have a limited effect, if any, while in flocculated systems pronounced changes can be brought about by the use of suitable dispersing agents.

Table I. Effect of Several Dispersing Agents on Some Representative Pigments

Pigment (20 Grams)	Dispersing Agent: Description	Dispersing Agent: Concentration	Wet Point	Flow Point	Difference	Rheological Behavior	Dispersion Quality	Pigment Concentration at Flow Point
		%	*Cc.*	*Cc.*	*Cc.*			*% by volume*
ZnO[a]	Water	..	6.3	20.9	14.6	Rises on tapping	Poor	14.9
	Dialyzed waste sulfite liquor	2	5.3	6.1	0.8	Flows on tapping	Fair	37.5
		3	4.8	5.4	0.6	Flows	Good	40.3
		5	5.2	5.4	0.2	Flows	Good	40.3
		7	5.1	5.4	0.3	Flows	Good	40.3
TiO_2[b]	Water	..	9.0	16.9	7.9	Rises on tapping	Poor	21.8
	Dialyzed waste sulfite liquor	2	8.6	10.6	2.0	Flows on tapping	Fair	30.7
		3	8.5	9.8	1.3	Flows on tapping	Fair	32.4
		5	8.0	9.3	1.3	Flows on tapping	Fair	33.5
		7	8.3	9.7	1.4	Flows on tapping	Fair	32.6
	Sodium salt of polymerized polyaryl sulfonic acids	3	8.9	12.2	3.3	Rises on tapping	Poor	27.8
		5	8.4	11.8	3.4	Rises on tapping	Poor	28.4
Fe_2O_3[c]	Water	..	6.2	19.4	13.2	Rises on tapping	Poor	16.7
	Dialyzed waste sulfite liquor	5	6.1	7.1	1.0	Flows	Good	35.4
	Modified glucoside extract	5	6.1	9.3	3.2	Rises on tapping	Poor	29.5
$PbCrO_4$[d]	Water	..	6.4	13.7	7.3	Rises on tapping	Poor	19.5
	Modified glucoside extract	5	6.2	8.3	2.1	Flows	Good	28.5
	Sodium salt of sulfonated condensed naphthalene	5	6.2	10.0	3.8	Rises on tapping	Poor	24.9
Furnace black[e]	Water	..	24.7	35.7	11.0	Rises on tapping	Poor	23.7
	Modified glucoside extract	3	20.2	21.5	1.3	Flows	Good	34.1
		5	19.4	24.7	5.3	Flows on tapping	Fair	31.0
		7	19.2	29.9	10.7	Flows on tapping	Fair	27.1
Carbon black[f]	Water	..	34.6	67.4	32.8	Rises on tapping	Poor	14.1
	Modified glucoside extract	5	28.0	29.0	1.0	Flows on tapping	Fair	27.7
		10	28.8	30.8	2.0	Flows on tapping	Fair	26.5
$BaSO_4$[g]	Water	..	6.5	12.2	5.7	Rises on tapping	Poor	27.6
	Sodium salt of sulfonated condensed naphthalene	5	5.7	6.7	1.0	Flows	Good	41.0
Hansa yellow[h]	Water	..	19.2	45.8	26.6	Rises on tapping	Poor	23.9
	Dialyzed waste sulfite liquor + wetting agents	5	17.0	19.8	2.8	Flows	Good	42.1

[a] Zinc oxide, lead-free, 592 American Process, New Jersey Zinc Co.
[b] Titanium dioxide, pure, rutile, 350-OR, United Color & Pigment Corp.
[c] Iron oxide, precipitated, C. K. Williams & Co.
[d] Lead chromate, medium, C.P., United Color & Pigment Corp.
[e] Furnace black, Furnex, Binney & Smith Co.
[f] Carbon black, Raven 15 Black, Binney & Smith Co.
[g] Barium sulfate, precipitated, C. J. Osborn Co.
[h] Hansa Yellow, Horicon X-1351, Imperial Paper & Color Corp.

STEPS IN EVALUATION. *A. Which of a number of dispersing agents produces the best dispersion with a given powder?*

A single test with a solution of 5% concentration is generally sufficient to answer this question. The agent which requires the smallest amount of liquid to go from wet point to flow point and displays the highest degree of dilatancy for a given powder is, as a rule, the most powerful dispersing agent. Final judgment, however, should be reserved until at least three determinations at different concentrations of dispersing agent have been made.

In many cases, too, the total amount of liquid used at the wet point and flow point is a good measure of dispersion. The less liquid required for a given powder, the better the dispersion. When it comes to a close comparison of several good dispersions, not infrequently an agent requiring slightly less liquid to reach the flow point shows the wider gap between wet point and flow point and less dilatancy. Careful checks against other dispersion tests indicate that the size of the gap and the rheological behavior are more significant as to degree of dispersion at high powder concentration than the absolute amount of liquid required. On the whole, however, the rheological characteristics of a mixture conform to the concentration rule, according to which the better dispersion requires less liquid at the flow point than the poorer dispersion.

B. What is the minimum amount of dispersing agent needed for optimum dispersion of a given powder?

This is determined by making successive tests, each test with the dispersing solution at a different concentration. By going stepwise from excess strength to insufficient strength of solution and measuring in each case the amount of liquid used at the flow point, data are provided (Table I) for a curve in which the amount of liquid used is plotted as ordinate and the concentration of the dispersing agent as abscissa (see Figure 2).

The flow point curves show an inflection where the strength of the solution becomes insufficient to disperse all the particles, and sometimes they also bend upward away from the abscissa in the region of higher concentration of the dispersing agent. This means that in some cases an excess as well as a deficiency of dispersing agent can be detrimental to the quality of the dispersion, a fact which underlines the necessity of making a quantitative test in every instance and not drawing conclusions prematurely from a single test. This is clearly shown in Table I, which demonstrates the effect of several dispersing agents in varying concentration on some representative pigments.

Carbon black (Furnex from Binney & Smith) dispersed with a 7% solution of a modified glucoside extract shows passive dilatancy and fair dispersion and with a 3% solution, strong active dilatancy and good dispersion.

Where the curve runs through the minimum, or where it shows its point of inflection, there lies the "optimum"—i.e., at that concentration the best possible dispersion can be produced with the least amount of dispersing agent. (The corresponding wet point curve runs more nearly as a straight line, indicating that the wet point is almost the same for dispersed as for undispersed systems and therefore cannot be used to determine the optimum point. However, the wet point curve is significant as a point of reference. By plotting it along with the flow point curve, the quality of the dispersion can be seen at a glance. The better the dispersion, the closer the two curves approach each other, especially at the point of inflection. The absolute amount of liquid required at wet point or flow point is not characteristic and varies greatly from one powder to the next.) Sometimes there is no sharp point of inflection; instead, the curve turns upward gradually. In that case a decision must be made as to how much of the quality of the dispersion is to be sacrificed for economy's sake.

Calculations. With the data obtained from the curve one can easily calculate the percentage of dispersing agent required for best dispersion—for example, where 20 grams of powder are used in the test and the point of inflection lies at 5.4 cc. for a 3% solution, $\frac{5.4 \times 3.0}{100}$ or 0.162 gram of dispersing agent is required for 20 grams and 5×0.162 gram or 0.810 gram of dispers-

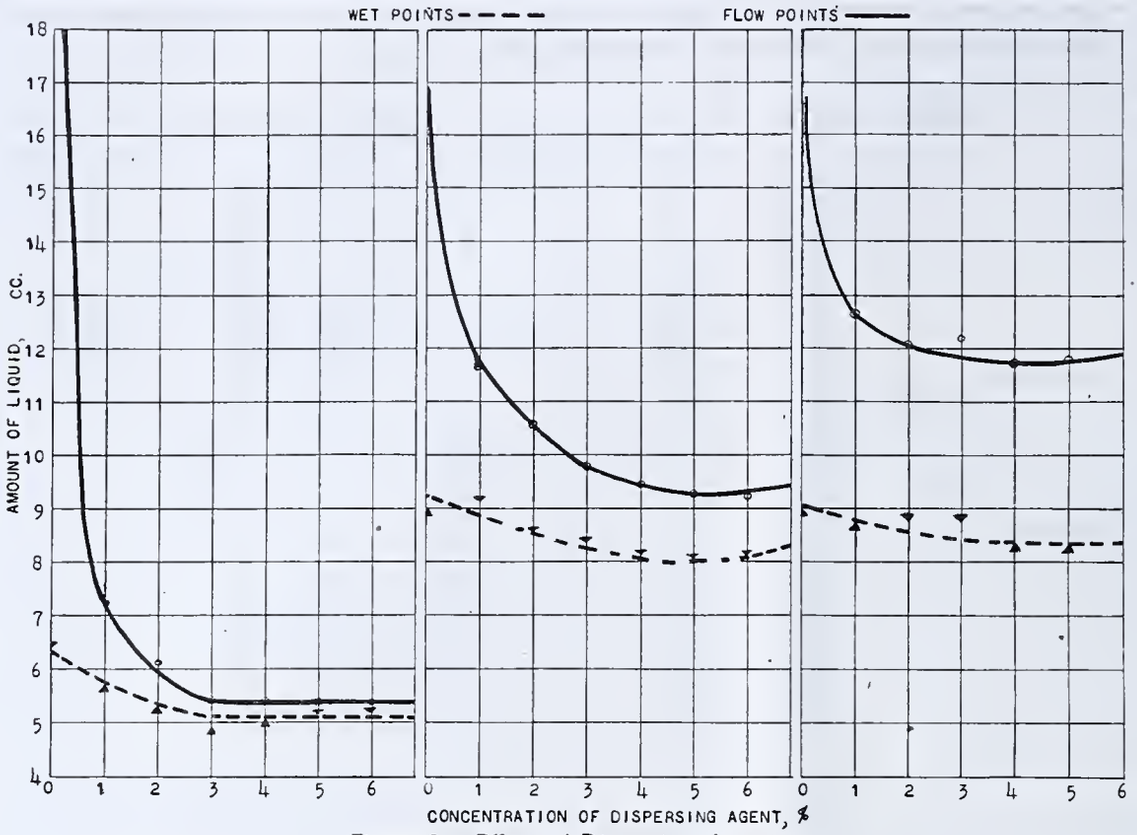

Figure 2. Effect of Dispersing Agents

Left. Good dispersion. 20 grams of ZnO dispersed with dialyzed waste sulfite liquor
Center. Fair dispersion. 20 grams of rutile TiO_2 dispersed with dialyzed waste sulfite liquor
Right. Poor dispersion. 20 grams of rutile TiO_2 dispersed with a sodium salt of polymerized polyaryl sulfonic acid

ing agent for 100 grams of the powder. For accurate calculations this last figure should be multiplied by the specific gravity of the solution, but for practical purposes, at the low concentrations used, the difference between milliliters and grams can be neglected.

C. What is the highest concentration of powder that can be obtained in a dispersion with a given dispersing agent?

This can likewise be calculated from the flow point curve.

Taking the amount of liquid, X, required at the optimum point for an amount of powder, Y, the concentration of powder in the dispersion is $\frac{100\ Y\ \%}{X + Y}$. In the example given above, the concentration would be $\frac{20 \times 100\%}{20 + 5.4} = 78.7\%$ by weight (40.3% by volume). In other words, 100 grams of dispersion of flow point consistency can be produced with 78.7 grams of powder, 0.637 gram of active dispersing agent, and 20.7 grams of water.

D. What is the effect of extraneous substances on a dispersion?

Dispersions are very sensitive towards extraneous substances. The adverse effect of electrolytes is well known (*2*). Polyvalent cations and acids especially will flocculate dispersions of the common type (negative charge). Not so well known, however, is the fact that many wetting agents also affect the dispersion detrimentally, while other combinations of surface-active compounds produce a greater dispersing effect than either one of the components. To make things still more complicated, such a beneficial combination may disperse nine powders very well and flocculate the tenth, although this one powder is chemically closely related to the others. In view of this unpredictable behavior of dispersions, it is essential to be able to determine the influence of each component separately.

The testing procedure is as follows:

Prepare about 50 grams of the dispersion at flow point consistency, using the dispersing agent which has been found most effective. Take a small part of that dispersion, add a few drops of one of the other liquid components in question, and mix with the spatula. If the rheological characteristics remain unchanged or the mixture becomes slightly more fluid because of the extra amount of liquid added, the compound has not affected the state of dispersion.

If the liquid added, preferably in concentrated solution, strongly liquefies the mixture, it has a dispersing effect. If, on the other hand, the dispersion becomes stiffer, dry, and loses its flow, the added substance detrimentally affects the dispersion.

In this way, the influence of each of the liquid components is quickly determined with a small portion of the original mixture and the formulation can be adjusted accordingly. This procedure has to be followed in all cases where the water-repellent nature of the powder calls for the use of wetting agents and where a good dispersion cannot be achieved with combination wetting-dispersing agents of known general compatibility. Under these conditions it will be necessary to prepare at least one dispersion of flow point consistency without a wetting agent and to try the effect of a number of wetting agents on it, as set forth above. To reach the wet point by the use of a dispersing agent alone takes considerably more time and effort than it does with pigments which are not water-repellent, but once reached, the wet point value is the same as that found so much more easily by the addition of wetting agents.

E. How shall dispersions consisting of more than one powder be tested?

A testing procedure similar to that used above may be followed. Dispersions of each of the component powders are made up singly at flow point consistency and then are combined one by one. If the combination results in loss of flow and causes the system to turn dull and stiff, the dispersion has been affected adversely. But again, the flocculated or flocculating component is clearly recognized and can be eliminated altogether, or, if that is not possible, a dispersing agent can usually be found which, though it may not be the most powerful dispersing agent for each component, avoids trouble in the complete formula.

Such testing procedure is unnecessary if sufficient dispersion data on the individual powders are available. The most suitable dispersing agent can be found by comparing the effect of each dispersing agent on each of the powders and selecting the one with the best over-all performance. This is demonstrated in Table II, in which the pertinent test data of four typical dispersing agents for zinc oxide and titanium dioxide are given.

While all four agents produce good dispersions of zinc oxide, D is more effective than A, B, or C as judged from the small gap between wet point and flow point. However, D produces a poor dispersion of titanium dioxide. Hence, where a dispersion of titanium and zinc oxide is called for, D is unsuitable in spite of its excellence as a zinc oxide dispersant. Agent B is obviously the one to be chosen for the best over-all result.

TIME REQUIRED. After standard solutions of the dispersing agents are prepared, each test should take no more than 7 to 10 minutes. Hence, the quantitative evaluation of a dispersing agent with respect to one powder can be carried out in about an

Table II. Variation in Dispersing Efficiency of Various Dispersing Agents Acting on Different Powders

Dispersing Agent, 5% Solution	20 Grams of ZnO			Dispersion	20 Grams of TiO_2			Dispersion
	Wet point	Flow point	Δ[a]		Wet point	Flow point	Δ[a]	
	Cc.	*Cc.*			*Cc.*	*Cc.*		
Agent A	4.7	5.2	0.5	Good	7.1	8.2	1.1	Fair
Agent B	4.7	4.9	0.2	Good	7.6	8.0	0.4	Good
Agent C	4.5	4.7	0.2	Good	8.4	11.8	3.4	Poor
Agent D	4.3	4.4	0.1	Good	8.2	12.2	4.0	Poor

[a] Δ = flow point minus wet point, cc.

hour's time, while a qualitative, or partial test as in A above can be made in just a few minutes.

PRECISION

Once the necessary technical skill has been acquired by an operator, which takes no more than a day or two, the quantitative experimental error becomes surprisingly small. For good dispersions, he can usually reproduce his results within a margin of 2%. For poor dispersions it may be as much as 5% and in some cases even slightly more.

The variation in results between two operators is also small. It depends primarily on how quickly, thoroughly, and energetically the mixing is performed, but different operators will never disagree as to the general character of the dispersion—that is, whether the dispersion is good, fair, or poor.

Tests were made with powders of widely different particle size, some with fine powders of an average particle size of 0.1μ and other tests with powders of an average particle size of 50μ. Within this particle-size range, the test proved to be fully applicable.

In view of the high reproducibility of the results, and considering the differences in the surface characteristics of different powders, it becomes understandable that the test can be used successfully for the rapid identification of pigments, and even for the identification of different modifications of the same pigment.

The test reveals the dispersing agent, among a number tested, which produces the best dispersion with a given powder or mixture of powders; the minimum amount of dispersing agent required to achieve optimum dispersion; the highest concentration of powder that can be obtained in a dispersion with a given dispersing agent; the effect of extraneous substances on a dispersion; and the effect of a mixture of powders on a dispersion.

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Separation of Acids by Chromatographic Adsorption of Their *p*-Phenylphenacyl Esters

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IN STUDYING the volatile flavor and odor constituents of pineapple (*5*), small amounts of ester mixtures were obtained which were difficult to separate sharply. Subsequently, the hydrolysis of these esters yielded mixtures of acids whose derivatives were in turn difficult to purify, especially when the quantity of available material was small. This necessitated the development of a method for separating small amounts of acids or their derivatives.

Various attempts have been made to separate the fatty acids directly by chromatography.

Cassidy (*1*) separated lauric, myristic, palmitic, and stearic acids on a carbon column from petroleum ether solution, and Swift *et al.* (*14*) purified methyl linoleate by passing cottonseed oil through alumina. All these separations involve colorless compounds, thus resulting in the consequent difficulties of separating the colorless bands. Cassidy (*1*) tried unsuccessfully to separate the *p*-aminoazobenzene derivatives of the higher fatty acids. Smith (*13*) separated formic, acetic, propionic, butyric, and valeric acids using an indicator to locate the bands on the column. In addition, Manunta (*10*), Kaufmann (*7*), Kaufmann and Wolf (*8*), Kondo (*9*), Papps and Othmer (*11*), Holmes and McKelvey (*6*), Rykhlikov (*12*), Elder and Springer (*3*), and Gyani and Ganguly (*4*) have made contributions in this field.

In searching for a method of purifying small amounts of acids or their derivatives, it was found that the highly purified *p*-phenylphenacyl derivatives (*2*) of the acids have a blue fluorescence in ultraviolet light. These derivatives can be adsorbed on silicic acid, resulting in the separation of acids differing by one CH_2 group, at least in the lower members.

Adsorption was made from a 1 to 1 benzene–petroleum ether (80° to 90° C.) mixture and development made with the same solvent. The refractive index of this mixture is such that it makes the adsorbent translucent (*15*).

The adsorbent used in this case was Merck's silicic acid (reagent grade). Different batches were found to vary in adsorption power. However, by evacuating a 10-cm. layer in a desiccator over phosphorus pentoxide for 4 minutes with a Hyvac pump, a fairly uniform product was obtained. Reactivation of the adsorbent was accomplished in the same manner after air-drying the acetone-eluted material.

The separations reported here were made with mixtures of derivatives, each of which had been previously chromatographed by itself to obtain a pure product. Derivatives which had been recrystallized only to a constant melting point still contained sufficient impurities to produce a yellow to white fluorescence.

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It was found advantageous to treat an alcohol solution of the derivatives with a decolorizing charcoal which adsorbed some highly fluorescent impurities that were removed with difficulty by adsorption on silicic acid. On chromatographing a charcoal-treated derivative under ultraviolet light, the white to yellow band which first appeared gradually developed, so that a blue band appeared beneath the more highly colored zone. This blue band was cut into sections and eluted with purified acetone. A bright fluorescent zone at the top of the column was due to traces of impurities which were strongly adsorbed. Any excess *p*-phenylphenacyl bromide was washed out ahead of the acid derivative.

For the separations 1.2 × 25 cm. columns were employed, using pressure to develop the chromatograms. The columns were wet with benzene–petroleum ether (80° to 90° C.) mixture and the derivatives added in a small quantity of the same solvent. This preliminary adsorption was accomplished with a pressure of 0.35 kg. per sq. cm. (5 pounds per square inch). The solvent mixture was then added and the pressure increased to 1 kg. per sq. cm. (15 to 20 pounds per square inch). The columns were equipped with a reservoir of solvent, so that there was no interruption during development.

The higher molecular weight derivatives were less strongly adsorbed than the lower members, so that a uniform series of separations was obtained from acetic to capric acids, inclusive. Each member could be separated from the one preceding or following it, the higher carbon member always being lower on the column. Similarly isobutyric, isovaleric, and isocaproic *p*-phenylphenacyl esters could be separated from one another and gave the same sequence. The capric and undecylic derivatives could not be separated, nor could the lauric and myristic derivatives.

Table I. Acid Mixtures Whose *p*-Phenylphenacyl Esters Have Been Separated by Chromatographic Adsorption

Acetic-propionic	*n*-Valeric–ethyl methyl acetic
Acetic-butyric	*n*-Valeric–trimethyl acetic
Acetic-valeric	Isovaleric–ethyl methyl acetic
Propionic-butyric	Isovaleric–trimethyl acetic
Propionic-caproic	Isovaleric-isocaproic
Propionic-heptylic	Caproic-isovaleric
Butyric-isobutyric	Caproic-heptylic
Butyric-valeric	Caproic–trimethyl acetic
Butyric-caproic	Caproic-caprylic
Butyric-isovaleric	Heptylic-caprylic
Isobutyric-isovaleric	Heptylic-capric
Isobutyric–*n*-valeric	Caprylic-pelargonic
n-Valeric–isocaproic	Caprylic-capric
n-Valeric–*n*-caproic	Pelargonic-capric

The separation of the lower members was characteristic. If a mixture of two of these chromatographically pure derivatives was placed on the column, there first appeared a whitish band which gradually narrowed as development continued with the appearance of blue bands above and below the white band. As the white band disappeared, separation was completed. In going further up the series this characteristic white band is not seen and the separations become less sharp. With the lower members the separation is quantitative.

To illustrate this, 16.9 mg. of butyric derivative and 17.1 mg. of acetic derivative were chromatographed on a column measuring 1 × 18 cm. After development the column under ultraviolet light consisted of the following bands: 2.5 cm. colorless, 2.5 cm. blue, 0.6 cm. colorless, and 2.5 cm. blue (listed from the top downward). From the upper blue band 16.3 mg. of acetic derivative melting at 110.5–111° C. were obtained. The lower blue band yielded 16.8 mg. of butyric derivative melting at 81.5–82° C.

In a similar manner, 27.2 mg. of acetic derivative and 21 mg. of *n*-propionic derivative were separated on a column measuring 1.2 × 25 cm. After development the upper 5-cm. blue band was separated from the 4.5-cm. lower blue band by a 1.9-cm. colorless zone. The upper blue band yielded 26.8 mg. of derivative melting at 110–111° and the lower band yielded 20.7 mg. of derivative melting at 102–103° C.

With the higher members, the adsorbed derivatives formed a continuous band. Sectioning of this with subsequent elution yielded a pure top and bottom section with the intervening sections showing various stages of mixtures.

The *n*-butyric derivative (m.p. 82° C.) can be separated from the isobutyric derivative (m.p. 89°). Ten milligrams of each of these were placed on a 1.2 × 25 cm. column, and after development the blue zone was cut into 7 equal sections. The eluted material from the two top sections melted at 78.6–80° and 78–78.9° C., respectively. On rechromatographing these the melting point was raised to 81–82°. The material from the lowest section of the first column melted at 85–87°, thus placing the iso derivative beneath the normal derivative. Isobutyric *p*-phenylphenacyl ester showed only a partial separation from the *n*-valeric derivative; the material from the upper part of the band melting at 82–84° compared to an expected value of 88–89.5° and that from the bottom part melting at 64–66° instead of 69° C. Although the *n*-valeric ester could not be separated from isovaleric ester, isovaleric ester was separated from the methyl ethyl acetic ester. In this case the latter appeared beneath the isovaleric. In turn methyl ethyl acetic ester gave a partial separation from that of *n*-valeric. Trimethyl acetic was separated from valeric, isovaleric, and *n*-caproic derivatives, and in each case appeared at the bottom of the blue zone.

Table I lists the separations which have been accomplished with this method. By using the method, it was possible to raise the melting point of the *n*-valeric derivative from 63.5° to 69° C. In addition, the *p*-phenylphenacyl ester of trimethyl acetic acid has been prepared for the first time. It melts at 113.1–114° C.

SUMMARY

The *p*-phenylphenacyl esters when highly purified have a blue fluorescence in ultraviolet light. These derivatives can be separated from one another on silicic acid. Each member from acetic to capric, inclusive, could be separated from the one preceding or following it, the higher carbon member always appearing at the bottom of the column. The iso acids with four, five, and six carbon atoms formed a similar series. The isobutyrate derivative could be separated from the *n*-butyrate, but the isovalerate could not be separated from the valerate ester. In contrast with this, methyl ethyl *p*-phenylphenacyl acetate was separated from the isovalerate. The trimethyl acetate was separated from the valerate, the isovalerate, and the *n*-caproate. Neither the capric and undecylic, nor the lauric and myristic derivatives could be separated.

By this method the melting point of the *p*-phenylphenacyl derivative of *n*-valeric acid has been raised from 63.5 to 69° C. The *p*-phenylphenacyl ester of trimethyl acetic acid melts at 113.1–114° C.

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Determination of Specific Surface by Permeability Measurements

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The effect of varying porosity on air permeability of powders has been investigated. The present experimental data indicate that the relation between permeability, specific surface, and porosity may be represented by the following modification of Kozeny's equation:

$$K = \frac{g(1+a)}{5\eta S_0^2} \times \frac{\left(\epsilon - \frac{a}{1+a}\right)^3}{(1-\epsilon)^2}$$

For certain pulverized materials, such as ground limestone, quartz, and portland cement, $\frac{a}{1+a}$ was found to be approximately 0.11.

THE industrial importance of the state of subdivision of many substances has led to the development of various methods of determining particle size. The permeability of beds of powdered materials has recently been investigated as a property by which the particle size or specific surface of such materials may conveniently be measured.

Carman (*5*) utilized the permeability to liquids, while Gooden and Smith (*6*), Lea and Nurse (*9*), and Blaine (*3*) used air as the permeating fluid.

The relationship, developed by Kozeny (*7*) and Carman, upon which this work is based is

$$K = \frac{g\,\epsilon^3}{5\eta S_0^2(1-\epsilon)^2} \qquad (1)$$

where K = permeability of the porous medium or apparent linear rate of flow (volume rate divided by bed area) per unit pressure drop (expressed as grams per sq. cm. per cm.) across the bed (cm.^{4} per gram sec.)
g = acceleration due to gravity, cm. per sec.^{2}
ϵ = porosity or fractional void of bed (dimensionless)
η = absolute viscosity of fluid, grams per cm. sec.
S_0 = specific surface of powder, cm.^{2} per cm.^{3}

Lea and Nurse (*9*) developed an apparatus for measuring the air permeability of powdered materials and modified Equation 1 to express specific surface directly in terms of the apparatus constants and the manometer readings:

$$S_w = \frac{14}{\rho_1}\left[\frac{\epsilon^3 A h_1}{(1-\epsilon)^2 LCh_2}\right]^{1/2} \qquad (2)$$

where S_w = specific surface, sq. cm. per gram
ρ_1 = density of material tested
A = cross-sectional area of bed of material
L = depth of bed
C = constant for flowmeter capillary
h_1 = manometer reading across bed
h_2 = manometer reading across capillary

Although Carman has presented evidence tending to show that these equations are valid, and that variations of permeability with porosity are represented by the porosity function, $\frac{\epsilon^3}{(1-\epsilon)^2}$, over a wide range, the work of Blaine (*3*) and that of the Working Committee on Fineness of A.S.T.M. Committee C-1 on Cement (*2*) as well as preliminary tests made in this laboratory have indicated that for fine powders the permeability changes disproportionately with the porosity function.

Experiments were made to determine the effect of varying porosity on the air permeability. The apparatus used was essentially similar to that described by Lea and Nurse (*9*). The permeability cell had an inside diameter of 1.69 cm., and was filled to a depth of 3.00 cm. The capillary tube had a constant $C = 2.64 \times 10^{-6}$. Correction was made for the resistance of the cell membrane, which was of the order of 1% of the resistance of the bed of powder, in the calculation of the ratio of h_1/h_2.

For any given powder and cell S_w, ρ_1, A, L, and C in Equation 2 may all be considered constant. Then Equation 2 may be written

$$\left(\frac{h_2}{h_1}\right)^{1/3}(1-\epsilon)^{2/3} = k\epsilon \qquad (3)$$

where $k = \left(\frac{14}{\rho_1 S_w}\right)^{2/3}\left(\frac{A}{LC}\right)^{1/3}$

If the porosity function holds and Equations 2 and 3 are valid, a plot of $(h_2/h_1)^{1/3}(1-\epsilon)^{2/3}$ versus ϵ should yield a straight line intersecting both axes at the origin. The data obtained on pulverized quartz powder of varying fineness are plotted in Figure 1, and the data obtained on several other materials are plotted in Figure 2. In general the points fall in straight lines intersecting the x-axis to the right of the origin. In the case of the pulverized quartz the intercept is independent of the fineness. These plots are entirely equivalent to those of Powers (*10*), who plotted $Q^{1/3} \times$

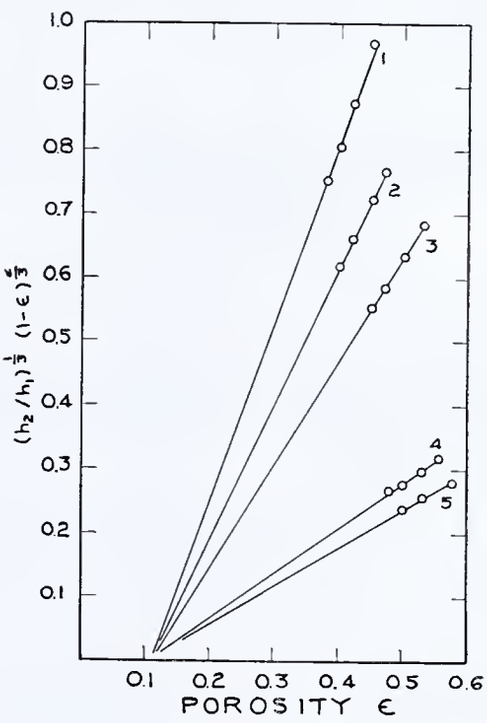

Figure 1. Permeability of Pulverized Quartz

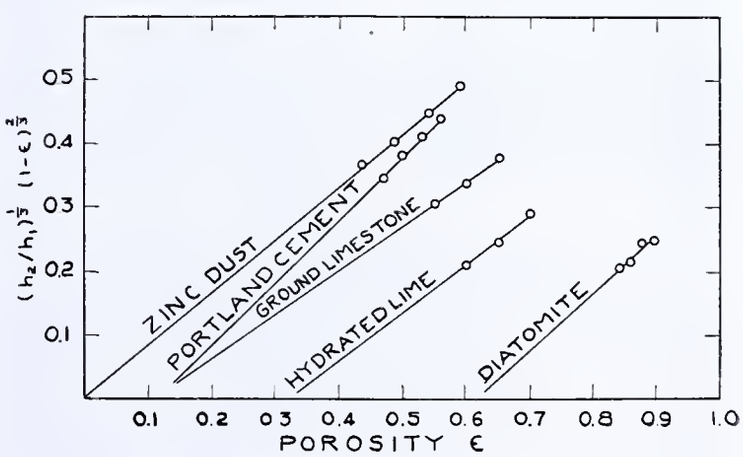

Figure 2. Permeability of Various Materials

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$(1 - \epsilon)^{1/3}$ versus ϵ, as Q, the volume rate, is proportional to the linear rate, and in the case of sedimentation the hydraulic gradient is proportional to the solids content $(1 - \epsilon)$. Powers found that the bleeding of portland cement pastes may be represented by a relationship based on Kozeny's equation if it is assumed that part of the fluid is immobile and does not flow. Steinour (*13*) has also demonstrated that the same relationship holds in the case of nonflocculated suspensions of emery powders and has shown the probable explanation of this phenomenon to be simple stagnation of fluid behind angular particles—that is, failure of fluid stream to trace out angularities of particles.

In view of the similarity of these plots with those of Powers it would appear reasonable to assume that the same inferences apply to the flow of a fluid through a bed of powder as apply to the sedimentation of powdered materials suspended in a fluid. Then, following the work of Steinour, if it is considered that the immobile fluid ϵ_i is proportional to the volume of solids—that is, $\epsilon_i = a(1 - \epsilon)$—then $\epsilon - a(1 - \epsilon)$ may be substituted for ϵ in Equation 1 to yield

$$K = \frac{g(1 + a)}{5\eta S_0^2} \times \frac{\left(\epsilon - \frac{a}{1 + a}\right)^3}{(1 - \epsilon)^2} \qquad (4)$$

Similar substitution in Equation 2 yields

$$S_w = \frac{14}{\rho_1}\left[\frac{(1 + a)\left(\epsilon - \frac{a}{1 + a}\right)^3}{(1 - \epsilon)^2} \times \frac{Ah_1}{LCh_2}\right]^{1/2} \qquad (5)$$

If, as before, it is considered that $\left(\frac{14}{\rho_1 S_w}\right)^{2/3}\left(\frac{A}{LC}\right)^{1/3} = k$,

Equation 5 may be written

$$\left(\frac{h_2}{h_1}\right)^{1/3}(1 - \epsilon)^{2/3} = k\,(1 + a)^{1/3}\left(\epsilon - \frac{a}{1 + a}\right) \qquad (6)$$

A plot of the experimental values for $(h_2/h_1)^{1/3}(1 - \epsilon)^{2/3}$ versus ϵ should yield a straight line. The slope of the line, $m = k\,(1 + a)^{1/3}$, the intercept is $a/(1 + a)$, and the specific surface,

$$S_w = \frac{14(1 + a)^{1/2}}{\rho_1} \times \left(\frac{A}{LC}\right)^{1/2} \times \frac{1}{m^{3/2}} \qquad (7)$$

The specific surface calculated in this manner represents the surface of the envelope separating the flowing from the immobile fluid. This surface may be somewhat greater than the true surface in the case of particles possessing no interior angles, or it may be substantially less in the case of particles possessing internal angles, pores, or fissures.

It will be seen that in the case of spherical zinc dust the intercept with the x-axis is the origin. This confirms Steinour's finding of no immobile fluid in the sedimentation of spherical particles, and indicates that in this special case Kozeny's equation is valid. In the case of certain pulverized materials, such as ground limestone, quartz, and portland cement, the intercept is $\epsilon = 0.11$ approximately. It is apparent that variations in shape of this class of particles are sufficiently small to have a negligible effect on the proportion of immobile fluid, and that differences in fineness have no effect. However, in the case of materials of high porosity, the intercept is greater than $\epsilon = 0.11$ and is varying. It is probable that in addition to immobile fluid due to the angularity of the particles there is also immobile fluid contained in those internal pores and fissures which communicated with the surface. The practical consequence of these variations of intercept is to require that permeability measurements on a given powder be made at sufficient porosities to establish the intercept unless it is already known.

Rigden (*11*) and Lea (*8*) believe nonuniform packing of the bed of powder to be the cause of the observed disproportionality of permeability to the porosity function. Although no proof to the contrary is available from the air-permeability experiments reported here, it is improbable that nonuniformity was a factor in Steinour's nonflocculated emery powder suspension. In addition, comparative fineness tests on portland cements using the apparatus described here and using the Blaine (*4*) apparatus gave excellent agreement when calculated by Equation 5 and the equivalent equation for the Blaine apparatus, regardless of the porosity of test. It is thought improbable that if nonuniform packing were present to any effective degree its magnitude should be dependent only on the porosity and not on the dimensions of the cell.

The possibility that the observed phenomena might result from simple adsorption of fluid on the surface of the particles is thought to be eliminated by the fact that the ratio of immobile fluid to solid volume was observed not to increase with increasing surface area and by the fact that the fluid layer of any reasonable thickness can be calculated to possess a volume too small to be detected by the methods used. Capillary attraction in the interstices may likewise be eliminated by the fact that no increase is observed with increasing surface area and by the absence of the effect in the case of spherical particles.

Table I. Specific Surface of Various Materials

Material	Surface *Sq. cm./g.*
Pulverized quartz 1	626
Pulverized quartz 2	958
Pulverized quartz 3	1430
Pulverized quartz 4	5000
Pulverized quartz 5	6330
Zinc dust	1400
Portland cement	2570
Ground limestone	5800
Hydrated lime	5800
Diatomite	6340

Table II. Comparison of Methods of Calculating Surface of Portland Cement

Method	Surface *Sq. cm./g.*	Ratio to Wagner Surface
Wagner turbidimeter	1870	...
Equation 2, $\epsilon = 0.47$	3670	1.96
Equation 2, $\epsilon = 0.50$	3510	1.88
Equation 2, $\epsilon = 0.53$	3410	1.82
Equation 2, $\epsilon = 0.56$	3350	1.79
Equation 7	2570	1.33

The specific surfaces of the materials shown in Figures 1 and 2 were calculated by Equation 7 and are given in Table I. The surface of the portland cement calculated from the data at each porosity by Equation 2 and that determined by the Wagner turbidimeter (*1*) is compared with that calculated by Equation 7 in Table II. Surfaces obtained by other sedimentation and elutriation methods by Roller and Roundy (*12*) and others are usually higher than those given by the Wagner turbidimeter by factors of from 1.2 to 1.5, and application of Equation 5 or 7 yields similar values.

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Factors Influencing Estimation of Free Fatty Acids in Dried Egg Powders

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At the normal pH of dried whole egg, fatty acids liberated during storage are incompletely estimated by the A.O.A.C. procedure for determination of acidity of the ether extract. In addition, the contribution of the extracted cephalin to the acidity of the ether extract may obscure significant percentage increases in the free fatty acidity of the egg powder. The necessity for drying the egg powder as required in the A.O.A.C. procedure is re-emphasized.

A CURRENT study in this laboratory of lipolytic activity in stored dried egg powders necessitated the use of a specific method for measuring glyceride hydrolysis. The method of the Association of Official Agricultural Chemists for the acidity of the ether extract of dried egg powder (*2*, page 314) has been generally used for this purpose and the results obtained have been reported variously as free fatty acids or as increases in the acidity of the ether extract (*3*, *4*, *5*, *11*, *19*). Briefly, this method consists of drying the egg powder to constant weight at 55° C. and under a pressure not exceeding 125 mm. of mercury. Samples of about 2 grams each are extracted with anhydrous ether, the ether is evaporated, and the residual oils are weighed and dissolved in neutral benzene and titrated to the phenolphthalein end point with 0.05 *N* sodium ethylate. The preliminary drying of the powder has been deemed unnecessary and omitted by several investigators (*3*, *5*, *11*).

The data presented here show that the indiscriminate use of results thus obtained, as a measure of either lipolysis or free fatty acid content, may be misleading.

METHODS

Phosphorus was determined according to the method of Allen (*1*). The micro-Kjeldahl procedure was used for total-nitrogen determinations. Amino nitrogen was determined by the Van Slyke manometric method, with 3-minute reaction times; *d,l*-alanine (Merck's) standards were assayed with each run. The formol titration, carried out electrometrically with the Beckman Laboratory Model pH meter, was also used to evaluate amino nitrogen; ethanolamine (redistilled) standards assayed by this procedure were titrated to the extent of 82% and this correction factor was therefore applied. Choline was determined by the reineckate method as modified by Glick (*12*). The pH of dried egg powder was evaluated with the Beckman meter on an emulsion of 1 gram with 3 grams of boiled distilled water. Apparent moisture content of the egg powder was determined by the A.O.A.C. method (*2*, page 308).

RESULTS

INCOMPLETE RECOVERY OF FATTY ACIDS. To estimate the total splitting of glyceride linkages it is essential to determine the liberated fatty acid, whether present as the free acid or the salt. At the pH of freshly dried whole egg (8.5 to 9) it is conceivable that the fatty acids liberated during storage, being weak acids, would react with the egg buffers to form salts. These salts—e.g., sodium oleate—would not, of course, contribute to the acidity of the ether extract, and the result would be incomplete measure of lipolysis.

Table I. Recovery of Added Oleic Acid

Added Oleic Acid *Mg./2 g. egg*	Resultant pH (Liquid)	pH Dried Egg, 1 G. Egg + 3 G. H_2O	Acidity of Ether Extract *Mg. oleic acid/2 g. egg*	Recovered Oleic Acid *Mg./2 g. egg*	Recovered Oleic Acid %
0	7.7	8.9	15.10	...	..
9.7	7.4	8.7	20.65	5.55	57
23.8	7.2	8.4	29.95	14.85	62
37.7	7.0	8.0	39.00	23.90	63
	Adjusted pH (Liquid)				
0	4.5	4.5	22.7	...	..
9.5	4.5	4.5	32.1	9.4	99
23.4	4.5	4.5	45.3	22.6	97
37.7	4.5	4.5	58.5	35.8	95

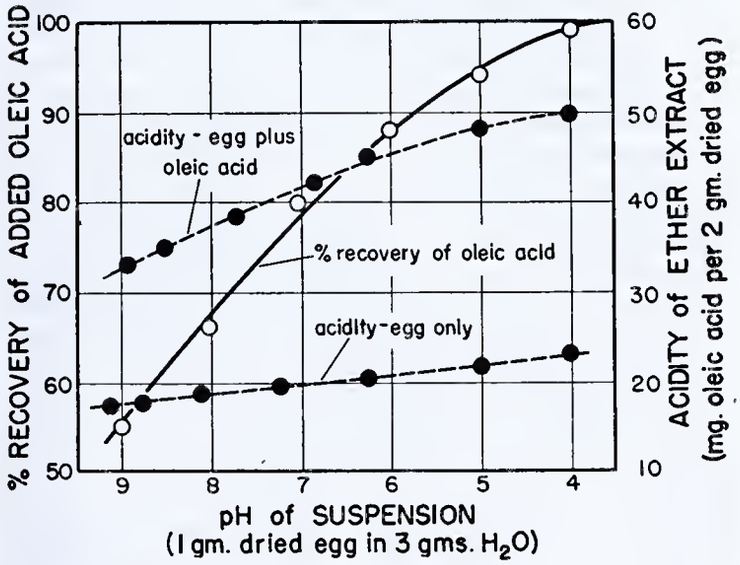


Figure 1. Relation between pH and Recovery of Oleic Acid from Dried Egg by A.O.A.C. Method

To determine the recovery of fatty acids as a function of the pH of dried egg, known amounts of oleic acid were added in ether solution to aliquots of whole fresh eggs, the mixtures were adjusted to various pH levels by the careful addition of 3 *N* hydrochloric acid, and the samples were lyophilized (dried in the frozen state under high vacuum). Oleic acid was used, since it has been reported to constitute about 50% of the egg fatty acids (*7*, *17*), and titration curves run in this laboratory have shown the mixture of egg fatty acids to have approximately the same pK as oleic. The dried egg powders contained 27 mg. of added oleic acid per 2 grams.

The oleic acid solution was checked against standard sodium ethylate. A set of controls, containing no added oleic acid, was prepared from the same batch of egg to cover the same range of pH. At least three 2.0-gram samples of each preparation were extracted with anhydrous ether for 4 hours in Soxhlet extractors and the acidity of the ether extracts was measured by the A.O.A.C. method.

The results are shown in Figure 1. The recovery of the added oleic acid at any pH was determined graphically by the difference in the acidity of the ether extracts of the control and the sample containing added oleic acid. Thus it was found that approximately 55 to 60% of the added oleic acid was recovered at the pH of freshly dried egg and that acidification below pH 5 was necessary to obtain essentially complete recovery.

That the pH change, which would be effected by the liberation of free fatty acid, is insufficient to increase the recovery appreciably is exemplified by the data in Table I, showing incomplete but slightly increasing recoveries with increasing amounts of added oleic acid. The recovery from samples prepared from the same batch of egg, but acidified to pH 4.5 prior to drying, is essentially complete.

DISTRIBUTION OF ACIDITY OF ETHER EXTRACT. From Figure 1 it is evident that the rate of increase of oleic acid recovery with

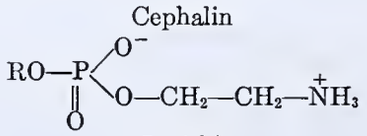
Table II. Analyses of Ether-Extracted Oils

Egg	H_2O	0.05 *N* Sodium Ethylate			Nitrogen			Phosphorus			Molecular Ratio, N/P			Oil Yield
		Whole oil	Acetone-soluble	Acetone-insoluble	Whole oil	Acetone-soluble	Acetone-insoluble	Whole oil	Acetone-soluble	Acetone-insoluble	Whole oil	Acetone-soluble	Acetone-insoluble	
	%	*Mg./g.*			*Mg./g.*			*Mg./g.*						%
Spray-dried A[a]	4.6	1.24	0.44	7.04	2.34	0.32	17.2	4.48	0.52	33.5	1.15	1.36	1.13	..
Spray-dried B[a]	3.7	1.41	0.48	7.16	2.92	0.39	18.6	5.12	0.56	33.4	1.25	1.55	1.23	..
Lyophilized[a]	1.7	1.45	0.43	8.44	2.26	0.24	16.0	4.62	0.42	33.3	1.09	1.27	1.06	..
A, stored[a]	5.6	4.00	3.72	6.32	2.22	0.59	15.9	4.58	1.14	33.6	1.07	1.14	1.06	..
Spray-dried A[b]	...	1.08	0.41	7.82	1.89	0.33	17.7	3.70	0.60	35.0	1.13	1.22	1.12	37.0
Spray-dried B[b]	...	1.22	0.46	7.06	2.52	0.38	18.8	4.48	0.62	33.9	1.24	1.35	1.23	37.1
Lyophilized[b]	...	1.22	0.41	8.01	2.01	0.33	16.0	4.05	0.55	33.3	1.10	1.33	1.05	37.7
A, stored[b]	...	3.60	3.33	5.95	1.93	0.56	15.6	3.93	0.96	33.6	1.09	1.29	1.03	36.8

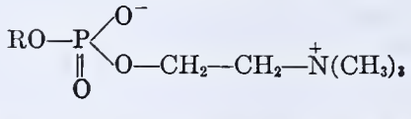
[a] Extracted without preliminary drying.
[b] Extracted after drying 24 hours at 55° C. and less than 3 mm. of Hg; residual moisture content, approximately 0.8%.

decreasing pH is considerably greater than the rate of increase in the acidity of the ether extract of the controls, thus indicating that the acidity value for freshly dried whole egg is, at least partly, attributable to some constituent other than free fatty acid. This interpretation is confirmed by reports in the literature and by experiments, described below, that point to egg cephalin as this constituent. That cephalins act as monobasic acids which are completely titrated in solvents of low dielectric value, with phenolphthalein as indicator, has been observed previously, mainly with animal cephalins (*9*, *13*, *18*). Jukes (*16*) found the alkali-binding capacity of egg phospholipid dissolved in 98% alcohol to be equivalent to the cephalin content as estimated by amino nitrogen determinations. Lecithin under similar titrating conditions has no alkali-binding capacity. The distinction between the ether-soluble egg phospholipids, lecithin and cephalin, lies in the nature of the base—choline in lecithin and ethanolamine in cephalin. The cephalin fraction of animal phospholipids has been reported to contain phosphatidyl serine and inositol in addition to phosphatidyl ethanolamine (*6*, *10*); Chargaff, Ziff, and Rittenberg (*6*), however, found no appreciable amino acid nitrogen in egg phospholipid. When written as the zwitterion structure, they may be represented, according to Fischgold and Chain (*9*), as follows:

Cephalin



Lecithin



The quaternary ammonium cationic group in lecithin is the ion of a very strong base and is not titrated in the A.O.A.C. method, whereas the primary amino group of cephalin is the cation of a weak base and is completely titrated to the $—NH_2$ form by sodium ethylate.

To determine the extent to which egg cephalin contributes to the acidity of the ether extracts, extracts prepared from four egg powders were analyzed. Two of these were commercially spray-dried products; one was prepared in this laboratory by lyophilizing whole-egg mix from several dozen Grade A fresh eggs; the fourth was a commercial spray-dried powder containing 5% moisture which had been stored at 98° F. for 9 months. Extractions were made at the moisture level of the samples as received, and also after a preliminary drying (Table II). Approximately 80 grams of each preparation were extracted with anhydrous ether in a Soxhlet extractor for a period extending 2 to 3 hours after the extracts appeared colorless. The extracts were centrifuged to remove any ether-insoluble material passing through the paper extraction thimbles. The ether was evaporated on the steam bath and traces of solvent were removed by drying in a vacuum oven at 70° C. and 3 mm. of mercury for 1 hour. To a 20-gram portion of each oil were added 180 ml. of ice-cold acetone and the turbid mixture was allowed to stand 2 hours in an ice-water bath. It was then centrifuged and the acetone-insoluble portion twice kneaded in 10-ml. portions of ice-cold acetone and centrifuged. The supernatant acetone solutions were combined and the solvent was removed from both fractions by evaporation on the steam bath, followed by vacuum-oven drying.

Yields, acidity, nitrogen, and phosphorus values for the whole oils and the fractions prepared therefrom are given in Tables II and III. The upper half of each table contains data for the eggs extracted without the preliminary drying. The acidities were determined by titration of benzene solutions to the phenolphthalein end point.

For the oils obtained from the three unstored egg powders, the acetone-insoluble fraction represents about 13% by weight of the original oil but contains about 70% of the acidity. This material is essentially phospholipid, as evidenced by the nitrogen and phosphorus contents and ratios. The slightly low nitrogen and phosphorus values are not unexpected, since impurities such as saturated triglycerides are removed with difficulty from the phospholipid by acetone trituration (*14*, *20*). The acetone-insoluble fraction from the stored egg powder represents 11% of the oil but contains only 17% of the acidity.

The acetone-soluble fractions, containing the free fatty acids, represent 26 to 32% of the original acidity for the unstored eggs, and 83% for the stored eggs, indicating large amounts of free fatty acid in the latter sample. Part of the acetone-soluble acidity may still be due to phospholipid, since about 10% of the nitrogen and phosphorus of the oils from the unstored egg powders and 23% for the oil from the stored sample remain in this fraction. The higher nitrogen and phosphorus content of this latter sample may be attributed to the increased solubility of the phospholipid in acetone imparted by the presence of large amounts of free fatty acid (*15*). It is difficult to account for the very considerable deviation of some of the nitrogen-phosphorus ratios from 1.0; however, as is shown later, these high ratios do

Table III. Distribution of Acidity, Nitrogen, and Phosphorus in Ether-Extracted Oils

Egg	H_2O	Yield of Whole Oil, %		Acidity, % of Total		Nitrogen, % of Total		Phosphorus, % of Total	
		Acetone-soluble	Acetone-insoluble	Acetone-soluble	Acetone-insoluble	Acetone-soluble	Acetone-insoluble	Acetone-soluble	Acetone-insoluble
	%								
Spray-dried A[a]	4.6	88.5	12.0	31	68	12	88	10	90
Spray-dried B[a]	3.7	85.9	13.9	29	71	12	88	9	91
Lyophilized[a]	1.7	87.2	12.8	26	74	9	91	8	92
A, stored[a]	5.6	89.3	10.7	83	17	23	77	22	78
Spray-dried A[b]	...	91.0	9.0	35	65	16	84	15	85
Spray-dried B[b]	...	88.7	11.6	33	67	13	87	12	88
Lyophilized[b]	...	89.4	10.7	30	70	15	85	12	88
A, stored[b]	...	90.8	9.1	85	15	26	74	22	78

[a] Extracted without preliminary drying.
[b] Extracted after drying 24 hours at 55° C. and less than 3 mm. of Hg; residual moisture content, approximately 0.8%.

Table IV. Alkali-Binding Capacity and Amino Nitrogen of Acetone-Insoluble Fractions

	Milliequivalents per Gram				% of Total N as NH_2N		
Egg	Sodium ethylate	Van Slyke NH_2N (hydrolyzed)	Formol NH_2N (hydrolyzed)	Van Slyke NH_2N (unhydrolyzed)	Sodium ethylate	Van Slyke (hydrolyzed)	Formol (hydrolyzed)
Spray-dried A	0.35	0.39	0.41	0.27	30	32	33
Spray-dried B	0.36	0.39	0.37	0.31	27	29	28
Lyophilized	0.42	0.44	0.44	0.40	37	38	38
A, stored	0.31	0.28	0.30	0.10	27	25	28

not invalidate the conclusions to be drawn as regards the contribution made by cephalin to the apparent acidity of the acetone-soluble oil.

The lower halves of Tables II and III show that drying prior to ether extraction results in appreciably lower acidities of the ether extracts. This result is correlated with the lower percentage of phospholipid in these oils. The effect of the moisture content of the egg at the time of extraction on the resulting acidity of the ether extract will be discussed in more detail later.

Alkali-Binding Capacity and Amino Nitrogen of Acetone-Insoluble Fraction. It has been determined that approximately 70% of the acidity of the ether extract from fresh egg powders resides in the acetone-insoluble (phospholipid) fraction of the extracted oil. Presumably, under the conditions of extraction and subsequent titration, the primary amino group of the cephalin constituent is responsible for the alkali-binding capacity, since no other reactive compounds have been reported in egg which would appear in this fraction. The amino nitrogen content was determined according to the method of Van Slyke, on weighed portions dissolved in glacial acetic acid and on aliquots of hydrolyzed material, and also by the formol titration method on the hydrolyzate.

For the hydrolysis, weighed portions of the phospholipid were refluxed with 2 *N* sulfuric acid for 48 hours; the fatty acids were removed by filtration and washed with hot 2 *N* sulfuric acid and the filtrate was made up to volume. The data given in Table IV show satisfactory agreement between the equivalents of alkali-binding capacity and amino nitrogen content as determined on the hydrolyzed phospholipid. Thus, for the acetone-insoluble fractions obtained from the unstored spray-dried powders, both the alkali-binding and amino nitrogen equivalents indicate that about 30% of the total nitrogen exists as cephalin. For the lyophilized egg sample, the value is higher, both measurements indicating a cephalin content of about 38%. The Van Slyke values on the unhydrolyzed phospholipids are lower, notably so for the stored egg powder. This finding is in agreement with the report of Chargaff *et al.* (*6*) that the amino nitrogen of various phospholipid preparations, including egg, decreased on storage, but that hydrolysis of the stored preparations gave considerably higher values. The agreement between the sodium ethylate equivalence values for the unhydrolyzed phospholipid and the amino nitrogen values for the hydrolyzed phospholipid suggests that the disappearance of the amino group may have been due to the formation of an amide that is easily hydrolyzed by alkali. Chargaff *et al.* (*6*) postulated amide formation in their study but did not test its lability to alkali.

Acidity of Acetone-Soluble Fractions. It has been noted (Table III) that about 10% of the nitrogen and phosphorus in the ether-extracted oils from the unstored egg powders remained in the acetone-soluble fractions. In order to correct the acidity of this fraction for nonfree-fatty-acid acidity, the cephalin content was estimated by amino nitrogen determinations and also by determining the difference between equivalents of total phosphorus and choline. The analyses were carried out on hydrolyzates prepared by autoclaving weighed portions of the acetone-soluble oils in 3 *N* hydrochloric acid for 4 hours at 250° F.; the fatty acids were removed with ether. From the average value of the two independent determinations (Table V) cephalin was found to represent about 23% of the acidity of the acetone-soluble fractions of the fresh egg oils, leaving the bulk of the acidity to be accounted for by free fatty acids. The acetone-soluble fraction from the stored egg powder contained less than 3% of its acidity as cephalin; the free fatty acid content was approximately 10 times that for the corresponding fractions for the unstored egg powders.

Converted back to the whole oil basis, the data of Table VI show that not more than about 22 to 26% of the acidity of the ether extract from fresh egg powder is due to free fatty acids.

Table V. Alkali-Binding Capacity of Acetone-Soluble Fractions

(Milliequivalents per Gram of Acetone-Soluble Oil) × 20

Egg	Choline	Total P − choline[a]	Formol NH_2N	Na ethylate	Approximate cephalin[b]	Net free fatty acids
Spray-dried A	0.27	0.12	0.085	0.41	0.10	0.31
Spray-dried B	0.29	0.11	0.080	0.46	0.10	0.36
Lyophilized	0.24	0.11	0.092	0.41	0.10	0.31
A, stored	0.55	0.07	0.081	3.33	0.08	3.25

[a] Total milliequivalents of phosphorus per gram of acetone-soluble oil minus milliequivalents of choline per gram of acetone-soluble oil taken to equal milliequivalents of cephalin; assumed that only negligible amounts of phospholipid other than lecithin and cephalin are present.
[b] Mean of values in columns 2 and 3.

Table VI. Summary of Acidity Fractionation

	Ml. of Sodium Ethylate (0.05 *N*) per Gram of Ether Extract	Acidity Due to			% of Acidity Due to		
Egg		Acetone-insoluble cephalin	Acetone-soluble cephalin	Free fatty acids	Acetone-insoluble cephalin	Acetone-soluble cephalin	Free fatty acids
Spray-dried A	1.08	0.70	0.09	0.28	65	8	26
Spray-dried B	1.22	0.82	0.09	0.32	67	7	26
Lyophilized	1.22	0.86	0.09	0.27	71	7	22
A, stored	3.60	0.54	0.08	2.97	15	2	83

Moisture Content and Acidity of the Ether Extract. Vacuum-oven drying of the four egg powders before extraction with ether resulted in appreciably lower acidities of the ether extracts and the data showed that this lower acidity was correlated with reduced phospholipid content of the oil. Additional data were obtained on one batch of egg powder adjusted to four moisture levels. A commercial spray-dried powder was dried in a vacuum oven at 55° C. with a pressure of about 3 mm. of mercury for 24 hours, resulting in a powder containing 0.86% moisture. Portions of this powder were adjusted to 0.31, 1.75, and 4.99% moisture by being held over Anhydrone, 71% sulfuric acid, and 51% sulfuric acid, respectively, in evacuated desiccators. Ether extracts were obtained as before. With increasing moisture content the acidity of the ether extract increases. This can be correlated with increased phospholipid extraction, as evidenced by greater acetone-insoluble nitrogen and phosphorus contents of the oils (Table VII). The acetone-soluble oil yield is not appreciably affected. Thus, if we assume that the increased phospholipid extracted contains cephalin, it is apparent that the increased acidities are attributed to increased extraction of phospholipid.

DISCUSSION

The incomplete extraction of liberated fatty acids results in low absolute values for increases in fatty acidity of the egg powder during storage or lipolysis studies. This source of error can be eliminated by reconstituting, acidifying to pH 4.5, and drying the egg in the frozen state under vacuum, prior to extracting the oil.

Table VII. Acidity and Composition of Ether Extract as Related to Moisture Content

	0.05 *N* Sodium Ethylate per Gram of Ether Extract	Dry Egg Basis		Whole Oil Basis			
H_2O		Acetone-insoluble	Acetone-soluble	Acetone-insoluble	Acetone-soluble	N	P
%	*Ml.*	%	%	%	%	%	%
0.31	0.93	3.5	33.2	9.5	90.5	0.178	0.352
0.86	1.08	3.5	32.7	9.8	90.2	0.195	0.362
1.75	1.17	4.0	32.7	10.8	89.2	0.204	0.382
4.99	1.28	4.7	33.1	12.4	87.6	0.240	0.458

In the measurement of the free fatty acid content of egg oil, and of animal lipoids and certain vegetable oils, the alkali-binding capacity of cephalin must be taken into account, since in many cases it constitutes the major portion of the acidity of the whole ether extract. This fact was recognized recently by Hutt and Weatherall (*15*) in estimating the free fatty acid content of commercial lecithins and by Fairbairn (*8*) in lipolytic enzyme studies on phospholipids. Acetone precipitation suffices to remove the greater portion of the cephalin and, if complete elimination of cephalin is deemed necessary, alcoholic magnesium chloride in conjunction with acetone can be used (*8*, *14*).

In egg oil, the cephalin contributes a large portion (60 to 70%) of the acidity of the whole ether extract, thus obscuring significant percentage increases in the free fatty acidity of the egg—i.e., the percentage increase in the acidity of the whole ether extract may be relatively small while there may be a significantly large increase in the free fatty acidity. For example, as shown in Table IV, the difference between the acidities of ether extracts of lyophilized (unstored) and stored egg was about 3-fold; correction for the cephalin titer increased this to greater than 10-fold.

Interpretation of the acidity of the ether extract is further complicated by the variable ether extraction of cephalin at different moisture levels (Tables II and VII). Difficulty in obtaining reproducible results on the same lot of egg powder at the same moisture level, due to inconsistent extraction of phospholipid, has been observed.

ACKNOWLEDGMENT

The authors are indebted to Julia S. Furlow for carrying out the Van Slyke amino nitrogen determinations.

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Determination of Carotenoids and Lipid Amine-Aldehyde Products in Dehydrated Egg

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A spectrophotometric method for the determination of carotenoid content and a fluorometric method for the determination of ether-soluble brown products in powdered eggs are described. The development of brown lipid substances in stored egg powders introduces errors into the direct photometric measurement of carotenoids; however, these errors are minimized by the spectrophotometric procedure described. Both determinations can be performed upon a single ether extract of egg powder.

Loss of carotenoid indicates oxidative degradation in stored egg powder (*1*). The concentration of fluorescent lipid amine-aldehyde products in egg powders is also of interest, since recent studies have found correlation coefficients between lipid fluorescence values and palatability of −0.79 to −0.98 (*9*).

In connection with the research program on the evaluation of miscellaneous egg powders in this laboratory, the quantitative methods described below were devised for determination of carotenoids and brown products. Previously published methods for spectrophotometric determination of carotenoids in dehydrated egg (*5*, *8*) have involved saponification and isolation of the carotenoids in the unsaponifiable fraction. This step has been desirable, since brown substances in lipid extracts of stored powders interfere with spectrophotometric determination of carotenoid. Brown substances, including the lipid amine-aldehyde products, can be separated from the carotenoids by saponification and solvents partition; the lipid amine-aldehyde products have been measured spectrophotometrically and fluorometrically in the acid aqueous portion of the saponifiable fraction (*1*, *3*).

In the present paper a combined spectrophotometric and fluorometric method is presented by which carotenoids and lipid amine-aldehyde products can be determined directly upon the ether extract of egg powder without recourse to saponification and solvents partition.

SPECTROPHOTOMETRIC METHOD FOR CAROTENOID CONTENT

The problems of spectrophotometric determination of carotenoids in ether extracts of stored egg powders can be illustrated by the absorption curves of Figure 1. The absorption curve of the sample stored for 9 months at 15° F. is hardly distinguishable from that of a freshly dehydrated egg. In the sample stored for 9 months at 98° F. the carotenoid maxima are barely visible when superimposed upon the broad general absorption band of brown substances. It is obvious that calculation of carotenoid content from the observed density at a single wave length, while justifiable in the case of the sample stored at 15° F., would intro-

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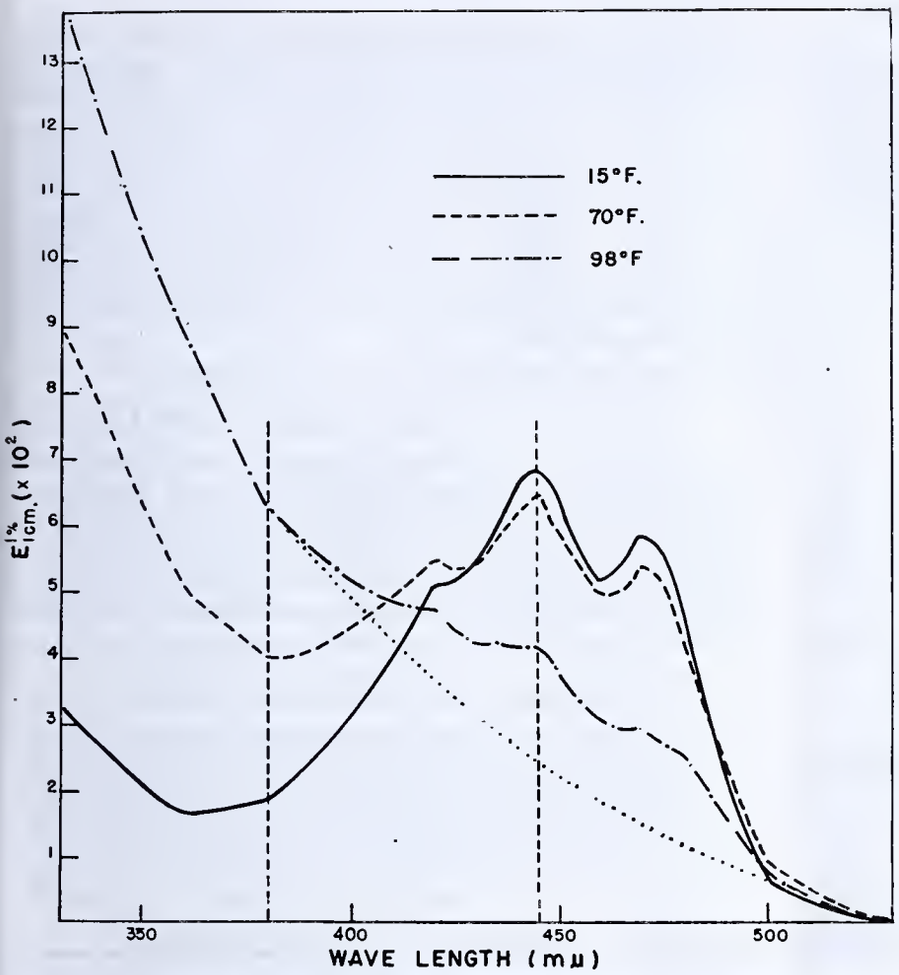

Figure 1. Absorption Spectra of Ether Extracts of Egg Powders Stored 9 Months

λ380 mμ and λ445 mμ are indicated by vertical lines. Dotted line is a hypothetical curve representing absorption due to brown products alone

duce large errors in the case of the sample stored at the higher temperatures.

The spectrophotometric method described here is based on the assumption that absorption by ether extracts of egg powders is due to two components, the carotenoid pigment and the brown substances which develop during storage. Each of these components is actually a complex mixture of substances of similar absorption properties. The simplification of considering the absorbing system as a binary mixture permits a rapid, reliable spectrophotometric determination of carotenoid content.

Density measurements are made at λλ 380 and 445 mμ, corresponding to a minimum and a maximum, respectively, in the carotenoid absorption curve. These densities can be expressed by the usual simultaneous equations for a binary mixture to which the Beer-Lambert law is applicable (*6*):

$$D^{445} = \alpha_x{}^{445} C_x l + \alpha_B{}^{445} C_B l \quad (1)$$

$$D^{380} = \alpha_x{}^{380} C_x l + \alpha_B{}^{380} C_B l \quad (2)$$

x and B refer to carotenoid and brown pigment, respectively; the numbers refer to the wave lengths; D, α, C, and l have their usual meanings of density, absorption coefficient, concentration, and path length. α as used here is defined by the equation

$$\alpha^{\lambda} = \frac{\log \left(\frac{I_0}{I}\right)_{\lambda}}{Cl} = \frac{D^{\lambda}}{Cl} \quad (3)$$

Since a path length of 1 cm. was used in these experiments, the solutions of Equations 1 and 2 for carotenoid content can be written

$$C_x = \frac{D^{445} - \left(\frac{\alpha_B{}^{445}}{\alpha_B{}^{380}}\right) D^{380}}{\alpha_x{}^{445} - \left(\frac{\alpha_B{}^{445}}{\alpha_B{}^{380}}\right) \alpha_x{}^{380}} \quad (4)$$

Since the absorption coefficients of the brown material appear in Equation 4 only as the ratio $\frac{\alpha_B{}^{445}}{\alpha_B{}^{380}}$, values for the individual coefficients are unnecessary. The ratio used is $\frac{D^{445}}{D^{380}}$ obtained on extracts of egg powders in which brown substances had developed and carotenoids had been largely destroyed. Correction is made for absorption due to the small amounts of remaining carotenoids by drawing through the region of carotenoid absorption a hypothetical curve (the dotted line of Figure 1) representative of absorption due to brown products only. The position of this curve on either side of the range of carotenoid absorption is given by the height of the total lipid extract curve; its shape conforms to what is known of the absorption curve of brown lipid materials in egg (*1*). The average value thus found for the ratio $\frac{\alpha_B{}^{445}}{\alpha_B{}^{380}}$ was 0.395 in six determinations which ranged from 0.37 to 0.45.

A slightly lower value for this ratio was obtained by the following method. The carotenoid contribution to total density at λλ 445 and 380 mμ was estimated as the total densities of the unsaponifiable fraction at these wave lengths (*1*). These densities were subtracted from the corresponding densities of the total extract to give densities due to brown substances alone, from which the ratio $\frac{\alpha_B{}^{445}}{\alpha_B{}^{380}}$ was calculated. However, the graphic method of correcting for residual carotenoid content is preferred because it is not influenced by any loss of carotenoid by isomerization or destruction during saponification.

The value used for the absorption coefficient, $\alpha_x{}^{445} = 255.5$, is Zscheile's for lutein in ethanol (*10*). Since the absorption of egg carotenoids in ether was found not to differ significantly from that in ethanol, the use of Zscheile's value would introduce little error. However, calculation of a coefficient for a 3 to 1 mixture of lutein and zeaxanthin as performed by Schrenk (*8*) would give a slightly lower result.

The coefficient α^{380} was estimated by measuring the ratio of densities $\frac{D^{445}}{D^{380}}$ on various freshly dehydrated egg samples containing no brown substance. The average of 25 determinations was 3.65. By combination of this average with the figure above for $\alpha_x{}^{445}$ the value $\alpha_x{}^{380} = 70$ is obtained. Substitution of these numerical values into Equation 4 gives finally the carotenoid content in grams per liter.

$$C_x = \frac{D^{445} - 0.395\ D^{380}}{227.8} \quad (5)$$

Solution of Equations 1 and 2 for C_B is not useful at present, since there is no satisfactory method of finding absolute values of the absorption coefficients $\alpha_B{}^{445}$ and $\alpha_B{}^{380}$. However, relative values of the concentration of brown substances can be found by using Equation 2 (with $l = 1$ cm.) in the form

$$D_B{}^{380} = \alpha_B{}^{380} C_B = D^{380} - 70\, C_x \quad (6)$$

Investigations made for the purpose of evaluating this method have shown that the quantity $D_B{}^{380}$ increases during storage at low temperatures while the fluorescence value described below remains constant. Thus the value $D_B{}^{380}$ probably does not measure the same material that is determined by the fluorescence value. The spectrophotometric procedure for determination of lipid amine-aldehyde products cannot be recommended therefore before the completion of further studies designed to improve its specificity.

FLUOROMETRIC METHOD FOR BROWN LIPID AMINE-ALDEHYDE PRODUCTS

Previous work has demonstrated that the lipid amine-aldehyde products are primarily responsible for the fluorescence observed in ether extracts of dehydrated egg (*1*). Development of a fluorometric method for estimating these products requires some general information about fluorometric behavior of egg extract as well as a practical technique for controlling extraneous fluorescence.

In the development of the procedure, the influence of two factors upon the fluorescence value of extracts of egg lipids has been studied: the effect of variable carotenoid content in lipid extracts and the effect of dilution upon the measured fluorescence value. In the procedure herein described the fluorescence value is defined as fluorescence of solution minus fluorescence of solvent on a scale such that fluorescence of the standard (0.4 microgram of quinine sulfate per ml.) equals 100.

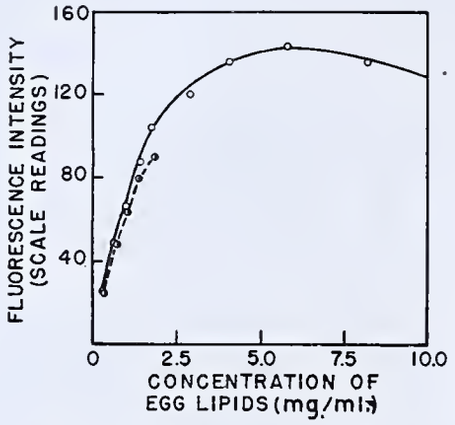

Figure 2. Change in Fluorescence Value with Concentration of Brown Lipids from Stored Egg

Open circles represent ether solutions of stored egg lipids. Half-filled circles show amount of deviation caused by introduction of crystalline α-carotene in concentration equivalent to that of carotenoid in extracts of average unstored egg (approximately 50 μg./gram of powder)

Table I gives fluorescence values of a standard egg oil solution to which increasing amounts of α-carotene have been added. (The oil, obtained by ether extraction of freshly lyophilized egg, had become highly discolored and had lost all its carotenoid after being stored for 11 days at 145° F.) Since carotene has approximately the same molecular weight and the same absorption properties as lutein, the chief carotenoid of egg, the data in Table I provide a basis for estimating the validity of fluorescence values obtained in the presence of variable amounts of carotenoid pigment. The solution of 0.4 microgram per ml. of carotene corresponds to the extract of an egg with extremely high pigmentation. It is apparent from the table that the presence of egg carotenoid does not invalidate fluorescence measurement.

In Figure 2 the open circles show the change in fluorescence with concentration of the egg oil, which represents the concentration of brown lipid amine-aldehyde material. Fluorescence intensities below 50 are in approximately linear relation with concentration of fluorescent material. Above this value deviations from linearity increase and the curve goes through a maximum. The curve, carried only to 10 mg. per liter in Figure 2, continues to fall; for a concentration of 145 mg. per liter the fluorescence is only 20. Similar curves for other fluorescent materials have been published (*4*). It is therefore evident that this fluorescence method must be considered strictly empirical; the ratio of grams of egg extracted to volume of fluorescent solution must be held constant.

COMBINED SPECTROPHOTOMETRIC AND FLUOROMETRIC PROCEDURE

All the glassware used, including the Soxhlet condensers, must be frequently and thoroughly cleaned with dichromate-sulfuric acid. The thimbles and cotton are ether-extracted for 2 hours before use, dried, and kept free from dust. Fluorescence of ether is reduced to a minimum by redistillation after refluxing over a lead-sodium alloy. (Because of its high fluorescence, commercial anhydrous ether is not a suitable solvent.) In this way extraneous fluorescence is reduced to a low, constant value.

Diethyl ether was selected as a solvent because (*a*) extraction for 4 hours in the micro-Soxhlet apparatus gave better spectrophotometric reproducibility than any grinding, shaking, or stirring technique; (*b*) a low-boiling solvent is required in this procedure in order to minimize heat-induced change in the egg.

One gram of dehydrated egg is extracted with anhydrous ether (prepared as described above) for 4 hours in a micro-Soxhlet extractor. The extract is diluted to 25 ml. with anhydrous ether and the optical density determined spectrophotometrically at wave lengths 380 and 445 mμ in a cell of 1-cm. path length. (The Beckman quartz spectrophotometer model DU was used in this study.) Carotenoid content of the egg powder is calculated by the following formula, which takes into account the dilutions specified:

$$\text{Micrograms of carotenoids per gram of egg powder} = 110\,(D^{445} - 0.395\,D^{380}) \quad (7)$$

For determination of the lipid fluorescence value, 1 ml. of the 25-ml. extract described above is diluted to 10 ml. with anhydrous diethyl ether. The fluorescence intensity is determined with the aid of the 365 mμ line of mercury for excitation—e.g., Coleman electronic photofluorometer with filters B_1 and PC_1—subtracting the fluorescence of the ether blank from the reading, and using quinine sulfate as fluorescence standard (0.4 μg. per ml. dissolved in 0.10 *N* sulfuric acid).

EVALUATION AND APPLICATION OF METHODS

Data concerning reproducibility of results are shown in Table II. Replicate analyses were made on 6 samples of egg representing a wide range of carotenoid and fluorescence values. The standard deviation, σ, for carotenoids expressed as per cent of the mean tended to diminish slightly with increasing concentration of carotenoid. σ for carotenoids is not affected by the quantity

Table I. Effect of Added Carotene on Fluorescence of a Discolored Egg Oil in Ether Solution

Concentration of Carotene μg./ml.	Fluorescence	Concentration of Carotene μg./ml.	Fluorescence
0	96	0.4	86
0.1	93	0.5	85
0.2	89	1.6	65
0.3	87	2.7	54

Each liter of solution contained 1.74 mg. of an oil obtained by ether-extraction of freshly lyophilized egg and stored 11 days at 145° F.

Table II. Reproducibility of Spectrophotometric and Fluorometric Methods

Sample No.	H_2O, %	No. of Replicates	Spectrophotometric Carotenoid content, mean μg./gram dried egg	Spectrophotometric σ as % of mean	Fluorometric Lipid fluorescence value	Fluorometric σ as % of mean
1	5	6	9.48	4.08	75.4	9.75
2	2.5	8	11.1	2.85	40.2	5.48
3	0.7	7	28.3	3.78	22.0	8.54
4	2.6	8	53.3	4.20	40.6	7.11
5	2.5	6	60.0	4.82	3.10	13.98
6	5	6	108	5.04	7.08	2.53
			Av. % of mean	4.13		7.90

Table III. Application of Spectrophotometric Method to a Storage Experiment

Storage Conditions Temp.	Duration	Spectrophotometric Method—Carotenoids Direct (λ 445)	Chemical fractionation (λ 445)	Binary (λ 445, 380)	Difference from Chemical Direct method	Difference from Chemical Binary
° F.	*Months*	*μg./gram dried egg*	*μg./gram dried egg*	*μg./gram dried egg*	%	%
15	1	29.3	25.3	28.9	13.4	12.4
15	6	27.4	24.7	27.2	9.8	9.2
15	9	28.7	25.7	28.6	10.5	10.1
70	1	26.35	23.4	26.8	11.4	12.7
70	3	27.3	23.3	25.7	14.6	9.3
70	6	25.2	21.2	22.3	16.0	5.0
70	9	25.3	18.2	21.2	28.1	14.2
98	1	27.5	20.95	21.7	23.8	3.4
98	3	30.3	19.8	21.15	34.6	6.3
98	6	29.2	18.0	19.2	38.2	6.3
98	9	18.0	9.2	9.5	48.8	3.1

of fluorescent material or by moisture level. Since σ for fluorescence intensity is higher than for carotenoid concentration, the fluorometric method is obviously less reproducible than the spectrophotometric method for carotenoids.

Spectrophotometric determinations of the carotenoid contents of dehydrated egg samples by three different procedures are compared in Table III. The values in the column labeled "Direct" were obtained by measurement of optical density at λ 445 mμ of the total ether extract on the assumption that all absorption at this wave length was caused by carotenoid. The values labeled "Chemical Fractionation" were found in the same manner, except that measurements were made on the unsaponifiable fraction of the ether extract. The values labeled "Binary" were obtained from optical density measurements at λλ 445 and 380 mμ on the total ether extract and use of Equation 5.

Divergence between results by the direct method and the chemical method is due to at least two errors. These are the positive error inherent in the direct method due to absorption by brown substances in the lipid extracts and the negative error in the chemical method due to loss or destruction of carotenoids during the saponification procedure. Samples low in brown products (storage temperature 15° F.) show only the effect of saponification losses in carotenoid. With these samples, therefore, the binary procedure herein described will prevent carotenoid destruction by avoiding saponification and will produce results comparable to those obtained by the direct method. The other egg samples, which have developed brown discoloration, are subject to both the absorption error and the saponification error. Since these two types of error are eliminated by the binary method, it thus has the advantages of both accuracy and rapidity.

Although the spectrophotometric and fluorometric procedures have been described as a combined method to be used on the same extract, either determination can be made separately or in connection with any other procedure that may be of interest. The ether-extracted egg powder residue, for instance, is suitable for obtaining additional information by means of reflectance and salt fluorescence procedures (*7*, *9*). Some results obtained by the use of the latter techniques will be published separately (*2*).

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Spectrophotometric Method for Estimating Gossypol in Crude Cottonseed Oil

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Spectral absorption curves of the dianilino derivative of pure gossypol and that of the gossypol in an expeller and hydraulic crude cottonseed oil are given. A rapid spectrophotometric method having the accuracy and precision of the gravimetric method for estimating the gossypol content of crude cottonseed oil is presented. The time required for the analysis is less than 2 hours.

THE gravimetric method for the determination of gossypol is based upon the reaction of gossypol with aniline to form a relatively insoluble dianilino derivative. The reaction, however, proceeds rather slowly; and, in the presence of cottonseed oil, the recovery of gossypol is not completely quantitative. Royce (*2*) has proposed a method in which the dianilino-gossypol is precipitated in the presence of pyridine. The precipitate contains two molecules of pyridine of crystallization, which is less soluble; consequently, the recovery of gossypol is improved, but 7 to 20 days are required for complete precipitation. The method has been modified by Royce and Kibler (*4*), and further modified by Royce, Harrison, and Deans (*3*) and by Halverson and Smith (*1*). The latter, with the use of heat and constant agitation of the mixture, were able to shorten the time required for precipitation to 72 hours, with an over-all time of 5 days required for the determination.

This paper presents a colorimetric method that requires about 1.5 hours for the determination of gossypol which has a precision approaching that of the gravimetric method. This method is based on the reaction of gossypol with aniline; but, in contrast with the gravimetric method, the dianilino-gossypol is soluble in the reagents used at the concentration required by the spectrophotometric method.

EXPERIMENTAL

A standard solution of pure gossypol was prepared by dissolving 50 mg. of pure gossypol in about 10 to 15 ml. of peroxide-free ethyl ether, adding 5 grams of Wesson oil, and removing the ether under reduced pressure while warming the flask in warm water not above 60° C. The Wesson oil solution was made to 500 ml. with Skellysolve B.

Aliquots of this solution were used for preparation of a standard transmittance-concentration curve and a spectral absorption curve. The spectral absorption curve was obtained by pipetting 2-ml. aliquots of this solution into each of two 25-ml. volumetric flasks. One of these aliquots was made to volume with Skellysolve B to be used as a blank for preparing the absorption curve. The color was developed in the other aliquot after diluting to 5 ml. and heating with 0.5 ml. of aniline for 40 minutes as described under Procedure. After diluting to volume, the spectral absorption curve 1, Figure 1, was determined with the Beckman spectrophotometer, using the hydrogen discharge tube.

An average value, for 13 determinations, of 0.621 for $\log \frac{I_0}{I}$ at 440 mμ was obtained for 0.2 mg. of pure gossypol in 25 ml. when the color was developed with aniline as described under Procedure and when square cuvettes with a depth of 0.999 cm. were used on the Beckman spectrophotometer. The $E^{1\%}_{1\text{ cm.}}$ value at 440 mμ is 776. The concentration of gossypol in grams per 100 ml. may be obtained from $\frac{\log \frac{I_0}{I} \text{ at } 440\ m\mu}{776 \times \text{cm. depth}}$. From this equation, the amount of gossypol in the aliquot of cottonseed oil taken for analysis may be obtained and the gossypol content calculated.

A spectral absorption curve was determined with the Beckman spectrophotometer for the gossypol contained in a crude cottonseed oil produced by the expeller process and in one obtained by the hydraulic process. These oils are designated as expeller and hydraulic oils throughout this paper. In order to compare the absorption curves of the gossypol obtained from these oils with pure gossypol, the observed $\log \frac{I_0}{I}$ values for the gossypol in these oils at the various wave lengths were multiplied by the ratio ob-

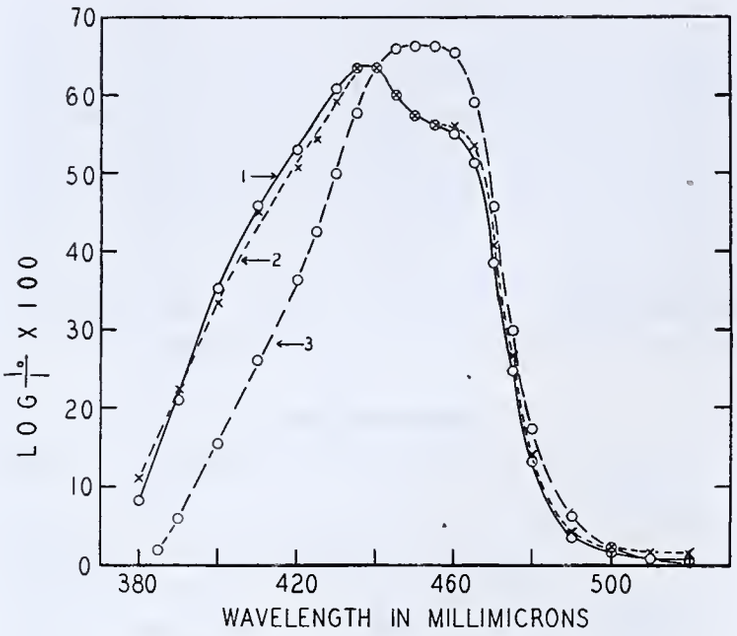

Figure 1. Absorption Curves of Aniline

1. Pure gossypol in Skellysolve B. 2. Expeller cottonseed oil in Skellysolve B. 3. Hydraulic cottonseed oil in Skellysolve B. In each case absorption read against respective untreated solution

tained by dividing the log $\frac{I_0}{I}$ value for pure gossypol at 440 mμ by the corresponding value obtained from the respective crude oil at this wave length. Curve 2, Figure 1, for the expeller oil follows that of pure gossypol, indicating that the material represented by curve 2 is gossypol. In the case of the hydraulic oil represented by curve 3, the maximum occurs between 445 and 455 mμ with a broad, rounded peak compared to the maximum between 435 and 440 mμ for the pure gossypol and the expeller oil. The absorption for the hydraulic oil is less than for the pure gossypol and the expeller oil in the region between 385 and 440 mμ and is about the same in the region from 470 to 520 mμ. The difference between the maximum of pure gossypol and the hydraulic oil is not sufficient to affect the analysis of gossypol by the spectrophotometric method. This shift of the curve to the right is similar to that occurring with extracts of cottonseed meal (*5*) and is probably due to a slight change in the molecule.

The standard transmittance-concentration curve was prepared by transferring three aliquots each of 0.2, 0.3, 0.5, 0.75, 1.0, 1.5, and 2.0 ml. of the standard gossypol solution to 25-ml. volumetric flasks. One flask containing each level of the standard gossypol solution was diluted to volume with Skellysolve B and used as a blank in the Coleman double monochrometer spectrophotometer. The aliquots in the other volumetric flasks were diluted to 5 to 6 ml. with Skellysolve B, and 0.5 ml. of freshly distilled aniline (water-white) was added; then the color was developed with aniline as described under Procedure. The per cent transmittance was read on the Coleman spectrophotometer at 440 mμ. The per cent transmittance plotted against concentration (0.02 to 0.20 mg. of gossypol in 25 ml.) on semilog paper gives a straight line and, therefore, obeys Beer's law. This line is represented by the following equation: concentration of gossypol in mg./25 ml. $= \frac{2.00000 - \log T}{5.171}$. A table may be prepared from this equation for converting transmittance readings to the corresponding weight of gossypol.

PROCEDURE

Filter the crude cottonseed oil under reduced pressure through a layer of washed Hyflo Super-Cel about 2 mm. thick placed over a disk of filter paper in a Hirsch funnel. (It is necessary to wash the Hyflo Super-Cel with hydrochloric acid to remove the traces of iron present, as iron destroys the gossypol. Boil 100 grams of Hyflo Super-Cel with 600 ml. of distilled water and 50 ml. of concentrated hydrochloric acid for 10 to 15 minutes, filter through a large Büchner funnel, and wash well with distilled water. Repeat the process and dry.) Prepare the layer of Hyflo Super-Cel by pouring a suspension of the Super-Cel in Skellysolve F or B over the paper disk while suction is applied. Discard the first few milliliters of oil filtered. The oil may be collected in a test tube placed in the suction flask. Pipet 5 ml. of the filtered crude oil into a 100-ml. volumetric flask, wiping the outside of the pipet with a clean cloth before adjusting to the mark. After draining, rinse the pipet into the volumetric flask with a stream of Skellysolve B from a wash bottle. Make to volume with Skellysolve B. Transfer two 3-ml. aliquots to 25-ml. volumetric flasks. Dilute one of the aliquots to volume with Skellysolve B to be used as the blank in the spectrophotometric determination of gossypol. Dilute the other aliquot to 6 ml. with Skellysolve B and add 0.5 ml. of freshly distilled aniline (water-white); then heat for 40 minutes on the metal top of the steam bath. Adjust the heating so only a small amount of steam is escaping from the bath. This causes only slight loss of Skellysolve B during the heating. This heating may also be done in a water bath at 60° to 65° C.

As the flasks are removed from the steam bath, add about 10 ml. of Skellysolve B to prevent the aniline from separating. Allow to cool, then make to volume with Skellysolve B and mix. Read the per cent transmittance on the Coleman double monochrometer spectrophotometer at 440 mμ, using the aliquot diluted with Skellysolve B as a blank. Scale the weight of gossypol in the 25 ml. from the standard curve or read it from the prepared conversion table. This value represents the weight of gossypol in the aliquot taken. The 3-ml. aliquot is equivalent to 0.15 ml. of oil. The specific gravity, 0.925, of cottonseed oil times the volume gives the weight of oil used in the determination ($0.925 \times 0.15 = 0.1388$ gram). The per cent gossypol is found by multiplying the weight of gossypol found in the aliquot by 100 and dividing by the weight of oil used.

The dianilino-gossypol color has remained stable for as long as 40 hours. Care must be exercised to prevent the blanks from becoming contaminated with aniline.

Three hydraulic oils were analyzed for the gossypol content by the gravimetric method of Halverson and Smith (*1*) and by the spectrophotometric method described above. Some of the determinations were made on the Beckman spectrophotometer, calculating the weight of gossypol from the extinction coefficient. The results are presented in Table I. There were no significant differences in the per cent gossypol found by the two methods. The variation in the gravimetric method is greater than that for the spectrophotometric method. The gravimetric method required 120 hours for completing the analysis, while the spectrophotometric method required less than 2 hours. This simple, quick method using common and inexpensive reagents is suitable for routine analyses as well as for more exacting work. When the concentration is too great to read on the spectrophotometer, aliquots from the blank and determination may be diluted with Skellysolve B to bring them within the reading range of the instrument.

The author is indebted to the Buckeye Cotton Oil Company, subsidiary of the Procter & Gamble Company, for the oil used in this investigation and to J. O. Halverson for suggestions.

Table I. Per Cent Gossypol in Crude Cottonseed Oil

Sample No.		Gravimetric	Spectrophotometric
		%	%
1		0.0168	0.016
		0.0177	0.016
		0.0168	0.017
			0.017
			0.017[a]
			0.016[a]
			0.017[a]
			0.017[a]
	Av.	0.0171	0.0166
2		0.0455	0.050
		0.0542	0.050
		0.0549	0.053[a]
		0.0518	0.053[a]
		0.0530	0.054[a]
		0.0524	0.053[a]
	Av.	0.0520	0.0522
3		0.0555	0.050
		0.0561	0.050
		0.0542	0.049
		0.0505	0.051
		0.0508	0.051[a]
		0.0528	0.051[a]
			0.052[a]
			0.052[a]
	Av.	0.0533	0.0508

[a] Determined by Beckman spectrophotometer.

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Estimation of Gossypol in Cottonseed Meal and Cottonseed Meats

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Spectral absorption curves are presented for the aniline derivative of pure gossypol and gossypol extracted from cottonseed meal and cottonseed meats with 60% alcohol containing ether in the Waring Blendor. These curves represent the difference between the absorption spectra of the aniline derivative of gossypol and of gossypol or gossypol extracts at the same concentration. The absorption curve for the cottonseed meats extract is practically identical with that of pure gossypol except in the region of 380 to 410 mμ. The curve for the cottonseed meal extract is shifted slightly to the right but is very similar to that of pure gossypol.

Cottonseed meal samples were extracted by allowing them to stand 10 minutes in 30% alcohol, then adding sufficient 72% alcohol to give a 60% alcohol to which ether was added. After blending for 5 minutes in the Waring Blendor, the extracts were filtered and made to 100 ml. Aliquots were taken for the blanks and determinations. The dianilino-gossypol color was developed in the latter by heating after adding aniline, then the per cent transmittance was read on the Coleman spectrophotometer, from which the gossypol content was determined. Duplicate determinations of gossypol in cottonseed meal or cottonseed meats may be completed in 2 hours with readily reproducible results.

A NUMBER of methods, both gravimetric (*2, 6, 7*) and spectrophotometric (*1, 5*), for the estimation of gossypol in cottonseed meal and cottonseed meats have been published. The gravimetric methods depend on the reaction of gossypol with aniline to form a relatively insoluble dianilino derivative. Lyman, Holland, and Hale (*5*) reported a colorimetric method based on this reaction. Boatner, Caravella, and Kyame (*1*) proposed a spectrophotometric method for estimating the gossypol content of cottonseed meal and cottonseed meats based on the reaction of gossypol with antimony trichloride. All these methods require a considerable period of time varying from 48 to 120 hours for completing the analysis. The amount of gossypol found depends upon both the extractant and the length of time the extraction is continued.

Three processes of extraction have been used: simple equilibration, intermittent flushing in the Soxhlet-type extractor, and continual washing in the Butt extractor. The gossypol content of cottonseed meal determined by the latter two methods depends somewhat on the moisture content of the meal and the amount of alcohol and water in the ether used for the extraction. In order to obtain satisfactory reproducible results, the methods cited must be followed with care. Moreover, the gossypol found in a given sample of cottonseed meal varies considerably when determined by the different methods.

A rapid method is proposed for determining the gossypol content of cottonseed meal or cottonseed meats in which the extraction is carried out in a Waring Blendor with 75 ml. of 60% ethyl alcohol containing 15 ml. of ethyl ether. The filtrate is made to volume and aliquots are taken for the spectrophotometric determination by a modification of the dianilino-gossypol method of Lyman, Holland, and Hale (*5*). This modification gives a more complete development of the dianilino-gossypol color which is stable for 24 hours or longer. Duplicate or triplicate determinations of gossypol in a sample of cottonseed meal may be completed in 2 hours; cottonseed meats require slightly less time. The results are readily reproducible. The composition of the extractant need not be held to extremely narrow limits. Values almost as high as those given in Table I were obtained with an 85% alcohol mixture. The 60% alcohol mixture has much better foaming characteristics, which gives a good suspension of the sample in the solvent and results in less splashing.

EXPERIMENTAL

In the work reported here, 25 mg. of pure gossypol were dissolved in 5 to 10 ml. of peroxide-free ether; most of the ether was removed under reduced pressure, and the solution diluted to 250 ml. with 95% alcohol. This, or a similar solution, was used for the standard transmittance-concentration and spectral absorption curves. Aliquots of 2 ml. of this solution were pipetted to two 25-ml. volumetric flasks. The color was developed in one of the flasks by heating the aliquot with 0.5 ml. of aniline for 40 minutes at 60° to 65° C., as outlined under Procedure, and the other was used as a blank for running a spectral absorption curve on a Beckman spectrophotometer, using the hydrogen discharge tube. This is represented by curve 1, Figure 1.

Three preparations of pure gossypol containing 0.2 mg. in 25 ml. gave an average value of 0.569 for $\log \frac{I_0}{I}$ at 445 mμ when the color was developed with aniline and read on the Beckman spectrophotometer using square cuvettes having a depth of 0.999 cm. The $E^{1\%}_{1\text{ cm.}}$ value at 445 mμ is 712. The concentration of gossypol in grams per 100 ml. $= \frac{\log \frac{I_0}{I} \text{ at } 445 \text{ m}\mu}{712 \times \text{depth in cm.}}$. The amount of gossy-

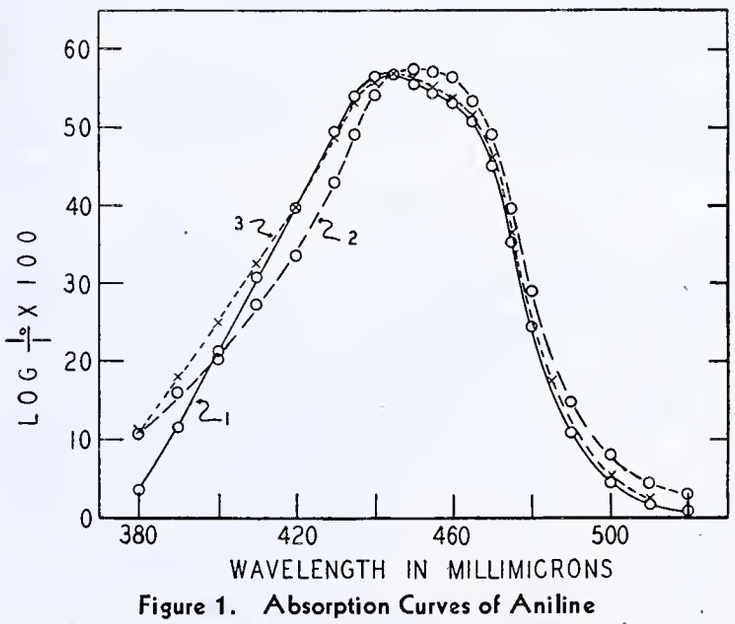

Figure 1. Absorption Curves of Aniline

1. Pure gossypol in alcohol-ether mixture. 2. Gossypol extracted from cottonseed meal with an alcohol-ether mixture. 3. Gossypol extracted from cottonseed meats with an alcohol-ether mixture. In each case absorption read against respective untreated solution

pol in the aliquot taken from the cottonseed meal or meats extract may be obtained from the above equation and the per cent gossypol content calculated.

Following the same procedure, a spectral absorption curve was obtained for the gossypol extracted from cottonseed meal by the proposed method. An absorption curve was likewise obtained for cottonseed meats. In order to compare these absorption curves with that of pure gossypol, the $\log \frac{I_0}{I}$ values observed at the various wave lengths of each extract were multiplied by the ratio obtained when the $\log \frac{I_0}{I}$ value of pure gossypol at 445 mμ was divided by the corresponding value of the respective extracts at this wave length. In this manner, curves 2 and 3, Figure 1, were obtained for the gossypol extracted from cottonseed meal and cottonseed meats, respectively. Curve 2 for the cottonseed meal is shifted slightly to the right with the maximum absorption at 450 mμ compared to 445 mμ for pure gossypol. A similar shift to the right occurs when hydraulic-expressed crude cottonseed oil is the source of the gossypol (*8*). This shift is so slight and the general character of the curve is so similar to that of pure gossypol in the region of 445 to 510 mμ that the absorption of the cottonseed meal extract in this region must be attributed to the gossypol present. This shift, however, may be due to a slight modification of the gossypol molecule. This divergence has no effect on the spectrophotometric estimation of gossypol in cottonseed meal. Curve 3, Figure 1, for cottonseed meats follows that for pure gossypol almost exactly in the region of 420 to 510 mμ. There appears to be slightly greater absorption in the region between 370 and 440 mμ for the aniline derivative of cottonseed meal extracts than for pure gossypol.

A standard transmittance-concentration curve was prepared by transferring three aliquots each of 0.2, 0.3, 0.5, 0.75, 1.00, 1.5, and 2 ml. from a standard gossypol solution containing 25 mg. of pure gossypol in 250 ml. of 95% alcohol, prepared as previously described, to 25-ml. volumetric flasks. Three preparations of pure gossypol were used for this curve. One of the flasks containing each aliquot was made to volume with 72% alcohol, by weight in water, to which 70 ml. of ether per 1000 ml. were added and used as a blank for the spectrophotometric determination. Five milliliters of the above alcohol-ether mixture and 0.5 ml. of freshly distilled aniline were added to each of the other flasks and the color developed as described under Procedure. The transmittance was read in the Coleman double monochrometer spectrophotometer at 445 mμ, using the blanks containing the corresponding amount of gossypol. The values were expressed as milligrams of gossypol per 25 ml. The standards follow Beer's law, giving a straight line when per cent transmittance *vs.* concentration is plotted on semilog paper. This line is represented by the linear equation: concentration in mg. of gossypol/25 ml. $= \frac{2.00000 - \log T}{4.846}$, in which T is the per cent transmittance. A conversion table giving the milligrams of gossypol in 25 ml. corresponding to the per cent transmittance may be calculated from the equation.

PROCEDURE

The extraction is carried out with the Waring Blendor using the small-size container, No. 17244, Central Scientific Company. The cardboard washer is removed from the screw cap and replaced with a washer cut from a sheet of rubber packing.

To a 2-gram charge of cottonseed meal placed in the Waring Blendor container, add 20 ml. of 30% (by weight) alcohol (384 ml. of 95% alcohol diluted to 1000 ml.) and allow to stand for 10 minutes. Rotate the jar two or three times by hand during this interval. Add 55 ml. of 72% (by weight) alcohol (830 ml. of 95% alcohol diluted to 1000 ml.) to give a mixture having an alcoholic content of 60% by weight. After adding 15 ml. of peroxide-free ethyl ether, blend for 5 minutes. Stop the blender and rinse down the walls of the container by swirling once during the period of blending. The cap should be loose enough to permit the expanding vapors to escape.

After blending, remove the cap, swirl the jar to suspend the residue, and pour into a 250-ml. beaker. Rinse the cap and jar with a stream of the alcohol-ether mixture (1000 ml. of 72% alcohol by weight to 70 ml. of ether), used for washing and making dilutions, from a wash bottle and transfer the washing to a second beaker to be used to wash the first beaker and residue after filtering through a filter tube (Corning 9480). Insert the filter tube and a bent glass tube for the application of suction in a two-holed rubber stopper placed in the top of a bell jar.

Prepare the filter by inserting a porcelain disk in the filter tube and, with vacuum applied, pour a layer of asbestos over it, followed by a layer of washed Hyflo Super-Cel about 2 mm. thick. (It is necessary to wash the Hyflo Super-Cel with hydrochloric acid to remove the traces of iron present, as iron destroys gossypol. Boil 100 grams of Hyflo Super-Cel with 600 ml. of distilled water and 50 ml. of concentrated hydrochloric acid for 10 to 15 minutes and filter, using a large Büchner funnel. Wash well with distilled water. Repeat the process and dry.) Suspend the washed Hyflo Super-Cel in 72% alcohol for preparing the filter. Receive the filtrate in a 100-ml. volumetric flask, containing 5 ml. of ether, placed under the bell jar. This ether replaces that lost during the filtration and prevents a slight turbidity due to the separation of oil from the mixture. Wash the first beaker and residue with the washings from the blender jar and then wash a second time with the alcohol-ether mixture from the wash bottle. Allow to cool and make to volume with the alcohol-ether mixture.

Table I. Gossypol Content of Cottonseed Meal and Cottonseed Meats

Sample No.		Gravimetric %	Blendor %	Lyman *et al.* %
2933		0.110	0.082	0.038
		0.118	0.083	0.038
		0.088	0.080	0.043
		0.109	0.088	0.037
		0.099	0.085	
		0.096	0.086	
	Av.	0.103	0.084	0.039
3039		0.117	0.077	
		0.103	0.081	...
			0.084	
	Av.	0.110	0.081	
3040		0.157	0.109	
		0.152	0.115	...
			0.118	
	Av.	0.155	0.114	
3041		0.100	0.072	
		0.096	0.074	...
			0.071	
	Av.	0.098	0.072	
3042		0.098	0.074	
		0.105	0.077	...
			0.071	
	Av.	0.102	0.074	
3043		0.099, 0.101	0.103	0.064
		0.092, 0.105	0.101	0.044
		0.107, 0.088	0.099	0.052
		0.106, 0.107	0.100	0.056
		0.111, 0.117	0.102	
		0.113, 0.116	0.100	
	Av.	0.105	0.101	0.052
3044		0.065, 0.059	0.053	0.025
		0.054, 0.053	0.052	0.026
		0.063, 0.051		
		0.067, 0.064		
		0.067, 0.070		
		0.061, 0.066		
	Av.	0.061	0.053	0.026
3073		0.095	0.098	0.040
		0.099	0.096	0.050
			0.102	0.055
				0.059
	Av.	0.097	0.099	0.051
3074		0.131	0.135	0.080
		0.123	0.128	0.080
		0.108	0.130	0.071
		0.103		0.083
		0.098		
	Av.	0.113	0.131	0.076
3096		0.087	0.058	0.051
		0.087	0.059	0.045
			0.061	
	Av.	0.087	0.059	0.048
3095		0.813	1.04	
		0.818	1.00	
		0.824	1.04	...
		0.818	1.04	
		0.827	1.03	
	Av.	0.820	1.03	

Two blender jars are advantageous, since one charge may be allowed to stand in the 30% alcohol while another one is being blended and filtered.

Use a charge of 0.2500 gram of cottonseed meats for the determination and mix the 20 ml. of 30% and the 55 ml. of the 72% alcohol before adding; otherwise a sticky paste is obtained which is not readily extracted. The 15 ml. of ether may be added directly to the jar. Blend the meats immediately, after which proceed as with cottonseed meal.

Transfer two 5-ml. aliquots to 25-ml. volumetric flasks. Dilute one of the aliquots to volume with 72% alcohol containing ether (1000 to 70 ml.) to be used as the blank in reading the transmittance of the gossypol with the spectrophotometer. Add to the other aliquot 0.5 ml. of freshly distilled aniline and heat on the metal top of the steam bath for 40 minutes. Adjust the steam so that only a small amount of steam is escaping. (This heating may be done in a water bath at about 65° C.) Remove from the steam bath, add 5 to 10 ml. of the 72% alcohol-ether mixture, and allow to cool. Then make to volume with the alcohol-ether mixture. Mix and read the intensity of color as per cent transmittance on the spectrophotometer at 445 mμ using the blank prepared from the extract. Take care to avoid contaminating the blanks with aniline.

The weight of gossypol in milligrams in 25 ml. may be scaled from the standard transmittance-concentration curve or read from the prepared conversion table. The value obtained is the per cent of gossypol in cottonseed meal. In the case of cottonseed meats, the milligrams of gossypol found per 25 ml. times 8 gives the per cent of gossypol.

The gossypol content of 10 samples of cottonseed meal was determined by the proposed method and compared with values obtained by the gravimetric method of Halverson and Smith as revised by Smith (*7*). Some of these meals were also run by the Lyman, Holland, and Hale colorimetric method. The results are shown in Table I. On the whole, the gravimetric values are slightly higher than those by the proposed method; however, the values for samples 3043, 3044, 3073, and 3074 are as high as those by the gravimetric method.

DISCUSSION

The mean difference in gossypol content as determined by the two methods was 0.0122 ± 0.0054% for all the samples of meals. The difference for individual samples of cottonseed meal, however, varied from 0.0408 to −0.0177%. This variation indicates that the ratios between the values of the two methods are not precisely the same for the different samples of meal. The variation for the gravimetric method for cottonseed meals 3043 and 3044 may be partially due to the use of different lots of ether for the extraction of the meal without modifying it to the optimum composition of 96.5% ether, 2.3% alcohol, and 1.1% water (*4*). All the meals used for the gravimetric method had a moisture content of 20 to 22%, which is the optimum for extracting gossypol from cottonseed meal by the method used (*3*, *4*, *5*).

The standard deviation for the proposed method was 0.0025% when the intensity of color was determined on two aliquots from the same extract. With one aliquot, the value would have been about 0.0027%, while one aliquot from each of two separate extracts would have had a standard deviation of about 0.0019%; therefore, it is recommended that two separate charges of cottonseed meal be carried through the entire process with the color being developed in only one aliquot from each extract. For control work involving large numbers of samples, however, single determinations should be satisfactory.

Gossypol contents of six of these cottonseed meals were determined by the method of Lyman, Holland, and Hale (*5*). The meals were extracted for 72 hours with peroxide-free ether containing 2.3% alcohol and 1.1% water (Table I). In all cases, the Lyman, Holland, and Hale method gave lower values than the proposed or the gravimetric methods; however, these authors have stated that higher values may be obtained if 20% moisture is added to the meal.

The value for cottonseed meats, No. 3095, by the proposed method was higher than by the gravimetric method, being 1.03 and 0.822%, respectively (Table I). However, 1.04 and 1.06% gossypol was found in these meats when they were extracted with ethyl ether as in the gravimetric method and the extracts subjected to the colorimetric procedure for oils (*8*) after removal of the ether and taking up in Skellysolve F. These values obtained for the ether extracts are practically identical with those obtained when the extraction was done in the blender, and are appreciably higher than those yielded by the gravimetric method. Boatner, Caravella, and Kyame (*1*) have reported that the antimony trichloride spectrophotometric method gives a higher value for some cottonseed meats than the gravimetric method. This indicates that some of the gossypol is not precipitated although it gives the color reaction with aniline. The spectral curve for this aniline derivative follows that of pure gossypol in the region of 420 to 520 mμ.

In the proposed method, the extraction is completed in 15 minutes with a mixture of alcohol, water, and ether (respectively, 51.6, 34.4, and 14.0%) in the Waring Blendor while 72 hours are required by the method of Lyman, Holland, and Hale using the Butt extractor with ether containing 2.3% alcohol and 1.1% water. The extract obtained by the proposed method is filtered and made to volume with the alcohol-ether mixture, as compared to the Lyman, Holland, and Hale method in which the ether is removed and the gossypol taken up in *N* butyl alcohol. These methods also differ in that the proposed method requires only one fourth as much aniline and that heat is used to promote the complete color development which is stable for a considerable time. In the Lyman, Holland, and Hale method, the color appears to develop more readily for pure gossypol than for that extracted from cottonseed meal. About 20 to 24 hours are required to reach the maximum color intensity by the latter. Ethyl alcohol solutions have advantages over *N* butyl alcohol from a manipulation standpoint.

The extraction was carried out on cottonseed meal No. 2933 with 75 ml. of alcohol adjusted to 56.5, 60, 70, 75, and 85%, and 15 ml. of ether after soaking in 24 or 30% alcohol for 10 minutes. The differences in the amount of gossypol found were very slight for the 56.5 to 75% alcohol, while that for the 85% was 0.014% lower than for the 60. This indicates that during the blending operation the concentration of the alcohol mixture may be permitted to vary a few per cent without affecting the results. Allowing the charge of cottonseed meal, but not cottonseed meats, to stand for 10 minutes before blending softens the meal, which facilitates the extraction of gossypol. Thirty per cent alcohol is more effective than 50 for this purpose.

The filtered extracts will beeome slightly turbid if too much ether is lost during the filtration. Aliquots used for the blanks will also become turbid when diluted to volume with a mixture of 1000 ml. of 60% alcohol and 70 ml. of ether. On the other hand, if the dilutions are made with 95% alcohol, turbidity frequently occurs. These difficulties are not encountered when all dilutions and washings are made with the 72% alcohol-ether mixture (1000 to 70 ml.) as recommended.

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Chemical Determination of Vitamin A in Mixed Feeds and Feedstuffs

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The antimony trichloride colorimetric reaction for determining vitamin A has been modified in order to correct for the color produced by nonvitamin A materials. The system of correction depends upon the action of light on the kinetics of the antimony trichloride reaction and the use of a preliminary chromatographic purification.

THE analysis of mixed feeds for their vitamin A content is complicated by the relatively low vitamin A level often present, the large number of different ingredients present in the mixture, and the wide variation in the physical and chemical characteristics of these ingredients. These complications have prevented the use of spectrophotometric methods for vitamin A measurement. The blue color reaction produced by vitamin A with antimony trichloride offers the best approach to the problem at the present time.

The antimony trichloride blue color reaction of Carr and Price (*3*) has high sensitivity to small amounts of vitamin A. This method as modified by Dann and Evelyn (*5*) has been widely used as the basis for a number of analytical methods adapted to a variety of materials. Oser, Melnick, and Pader (*7*) have used the blue color test in vitamin A measurements on food products in a procedure involving the use of an internal standard designed to correct for the presence of reaction inhibitors, temperature effects, reagent variations, turbidity, and extraneous color. Corbet, Geisinger, and Holmes (*4*) very early listed a large group of materials known to give a color reaction with antimony trichloride under conditions of the usual vitamin A test.

The reaction has been utilized for the quantitative measurement of vitamin D and some of the related sterols and the blue color produced by carotene and the xanthophyll pigments is common knowledge. Dann and Evelyn (*5*) attempted to correct for the reaction of carotene with antimony trichloride and the resultant interference with the vitamin A test. Oser, Melnick, and Pader (*7*) attempted to distinguish quantitatively between the blue color produced by the reaction of carotene and that produced by the reaction of preformed vitamin A by the difference in the intensity of the two color reactions with time. These authors found that the blue color of the reacted vitamin A faded almost completely in 2 hours, whereas the blue color of carotene increased in intensity over this period of time.

The present authors in an attempt to duplicate this work found that the period required for vitamin A blue color destruction was greatly influenced by both light and temperature conditions. The effect of temperature was noted by Norris and Church (*6*) and is a normal behavior, but the action of light has only recently been reported by Caldwell and Parrish (*2*) and appears to offer an explanation for some of the discrepancies in the vitamin A literature in regard to the rate of fading of the blue color.

Some types of mixed feeds and certain feed ingredients when reacted with antimony trichloride give strong color reactions of nonvitamin A origin that interfere greatly with the vitamin A tests. The action of light on the course of these nonvitamin A color reactions has been found to differ so greatly from the action on the true vitamin A blue color as to provide a means of differentiating between the color reaction of vitamin A and any interfering color reactions simultaneously produced.

ANALYTICAL PROCEDURE

REAGENTS. Antimony trichloride reagent, 90 grams of reagent grade antimony trichloride in 240 ml. of reagent grade chloroform.

Aqueous potassium hydroxide, 50 grams of reagent grade pellets in 50 ml. of distilled water.

Calcium phosphate, dibasic, should be tested to retain vitamin A and pass β-carotene (Mallinckrodt A. R. grade).

Skellysolve F, petroleum ether, boiling range 30° to 60° C.

EXTRACTION. Place a weighed 10-gram sample of feed in a 20 × 80 mm. paper extraction thimble and extract (all operations on vitamin A solutions should be carried out in subdued artificial light, preferably in amber glassware) for 4 hours in a Butt-type extraction tube with 35 ml. of Skellysolve F placed in a 100-ml. extraction flask. Other types of extraction equipment may require different volumes and extraction times.

Evaporate off the solvent from the extract under a vacuum, add 30 ml. of 95% ethyl alcohol and 3 ml. of aqueous potassium hydroxide solution, and bring to a boil in a water bath. Cool and wash contents of flask into a 250-ml. separatory funnel with a minimum amount of Skellysolve F. Add 30 ml. of distilled water and sufficient Skellysolve F to make 25-ml. total volume of the latter. Shake and draw off the lower phase after settling, and transfer the Skellysolve phase to a clean 125-ml. flask. Repeat the extraction of the aqueous phase with 3 more 25-ml. portions of Skellysolve and combine the extracts in a clean 250-ml. separatory funnel. Wash free from alkali by repeated washings or with a continuous spray washing device. Filter alkali-free extract into a 300-ml. long-necked round-bottomed flask through about 15 grams of anhydrous sodium sulfate on a filter paper, and wash the filter paper with additional solvent.

PURIFICATION. If little or no color is present in the final extract, this step may be omitted. If an appreciable amount of colored pigments is present, evaporate the extract down to a 5-ml. volume under a vacuum and wash into a chromatographic column with a minimum volume of Skellysolve.

Prepare a 16 × 150 mm. chromatographic column with a 7.5-cm. (3-inch) layer of dibasic calcium phosphate, topped by a 2.5-cm. (1-inch) layer of calcium carbonate. The dibasic calcium phosphate should be tested prior to use and shown to retain vitamin A without destruction and to pass β-carotene using Skellysolve F as a solvent. The Skellysolve should be free from ether, alcohol, or other solvents and should be as dry as possible.

After the extract passes into the column, wash at once with Skellysolve F and continue washing until all carotene passes through the column. This is indicated by a colorless filtrate. This solution may be retained and the carotene measured.

The appearance of the column at this point shows a sharp colored layer at the extreme top, usually followed by a series of lines and a broad colored band, all in the carbonate layer. Remove all these with care, except for the lowest portion of the broad colored band, by digging out the adsorbent with a suitable instrument.

Change the receiving flask on the column and elute the vitamin A by passing 25 ml. of ether slowly through the column or until all the remaining yellow lines pass through the column. This eluate is to be used for the vitamin A reading.

COLOR REACTION. Divide the eluate from the column, or the original extract if the column purification was not required, into two equal portions of any convenient volume. At this point it is desirable to estimate the approximate vitamin A content of the material being tested, in order that the final solution concentration of vitamin A will be approximately 15 units per ml., a concentration most suitable for measurement. A volume factor, x, is approximated from the formula:

$$x = \frac{(\text{approximate units of vitamin A per gram in feed}) \times (\text{weight of sample})}{30}$$

by which x is found to the nearest whole number (but not less than 2). To one portion of the solution add $10x$ units of a vitamin A solution (obtained from a dilution of Distillation Products vitamin A concentrate capsules, Control No. PC-3, checked spectrophotometrically). Evaporate both fractions to dryness under a vacuum and dissolve each fraction in exactly x ml. of dry chloroform. By this system one portion of the sample has been fortified with an internal standard equipment to 10 units per ml.

Reaction readings are made on the Evelyn photoelectric colorimeter, using a 620 mμ filter. Set a reagent blank containing 1 ml. of chloroform plus 10 ml. of antimony trichloride reagent to a reading of 100% transmission and find the corresponding reference no cell reading with blank solution tube removed. This

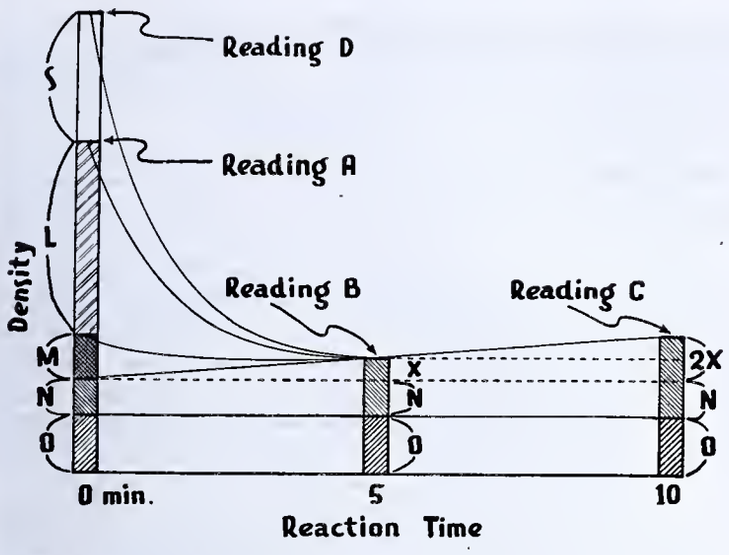

Figure 1. Derivation of Vitamin A Calculation Formula

reading is used for subsequent settings for a series of readings on any one day with the same reagent.

Place a 1-ml. sample of the unfortified unknown in a reaction tube in the instrument, add 10 ml. of antimony trichloride reagent, and take a reading as soon as the galvanometer can be read (reading A). Place the reacted solution immediately in a glass-walled water bath (a rectangular battery jar is suitable) kept at 30° C. midway between two 150-watt reflector flood lamps placed at a distance of 30 cm. (12 inches) between the two bulb faces.

After exactly 5 minutes read the reaction tube again (reading B) and immediately return it to the water bath. At the end of exactly 10 minutes obtain a third reading (reading C).

React a 1-ml. sample of the vitamin A–fortified sample as above with 10 ml. of antimony trichloride and take only the initial reading (reading D). According to the data of Baxter and Robeson (*1*), confirmed by the authors, this reading should not exceed a photometric density of 0.523, corresponding to a transmission of 30% in order to stay within the linear range for a vitamin A standard curve.

Dilute a second 1-ml. aliquot of the unknown (if necessary a part of the fortified sample can be mixed with the unfortified for this reading) with 10 ml. of chloroform and read at 440 mμ (reading E) against a blank of pure chloroform set to 100% transmission. Reading E is made to correct for the interference of certain carotenoid pigments which give color reactions with antimony trichloride. Calculate vitamin A concentration from the following formula in which all readings have been converted to photometric densities:

$$\text{Units of vitamin A per gram} = \frac{10(A - 2B + C - 0.067\,E)(2x)}{(D - A)\ (\text{wt. of sample})}$$

The factor 0.067 in this formula is explained below and should be determined by the user for the particular type of carotenoid pigments most commonly encountered (from alfalfa, corn gluten, etc.) and with the particular filters used in making the readings.

BASIS OF COLOR-READING PROCEDURE

The vitamin A blue color reaction with antimony trichloride is usually read at about 620 mμ within 10 seconds after addition of the reagent to the A source. Unfortunately, if the vitamin A solution contains any considerable amounts of impurities of a variety of types, these impurities will produce colored reactions simultaneously with the formation of the vitamin A blue color. The best available means of purification do not completely remove these interfering materials from feed extracts.

These interfering materials can be grouped into three broad reaction types:

1. Materials which produce a high initial color intensity which fades with time, similar to vitamin A itself. This is indicated by decreasing light absorption with time.
2. Materials which produce a low initial color intensity which increases with time. This is indicated by increasing light-absorption values.
3. Materials which produce a color reaction of an intensity relatively constant with respect to time. This is indicated by constant light-absorption values.

All classes of interference would be made up of one of these types or some combination of several types.

If vitamin A–antimony trichloride reaction solutions are subjected to controlled light of high intensity at constant temperature, as outlined in the quantitative procedure, the blue color fades very rapidly, and at the end of 5 minutes has completely disappeared. If reacted solutions of pure vitamin A are read at 620 mμ, the difference between the initial photometric density and that after 5 minutes is directly proportional to the A concentration over a wide range of concentration values.

Extracts of feedstuffs known not to be sources of vitamin A have been tested under similar conditions with the following observed groupings:

1. Reacting materials which showed decreasing color intensities with time were mostly carotenoid in nature and had attained minimum values of photometric density at 620 mμ within 5 minutes. Moreover, the difference between the initial absorption at 620 mμ and the absorption after 5 minutes in each case was quantitatively related to the yellow color present in the unreacted solution. The latter could be measured by reading at 440 mμ.
2. Materials which showed increasing color intensities with time increased in photometric density at approximately a uniform rate for short periods of time. The increase during the period 0 to 5 minutes was approximately the same as that for 5 to 10 minutes. In all cases this increase in photometric density at 620 mμ was relatively slow as compared with the rapid decreases observed for vitamin A and carotenoids and the difference between the changes in the two time intervals was negligible.
3. Materials which possessed a color of their own unrelated to the antimony trichloride reaction or which reacted to produce a color that did not change with short intervals of time.

Utilizing the facts ascertained in these observations, it was decided that a procedure could be developed for reading vitamin A in the presence of all three types of interfering materials. This is best explained by means of Figure 1.

Readings are made under controlled conditions of light exposure and temperature immediately after reaction (reading A), after 5 minutes' exposure (reading B), and after 10 minutes' exposure (reading C). In addition, a second portion of the extract being tested is reacted in the presence of a known addition of pure vitamin A (reading D) and an independent reading is made at 440 mμ (reading E) of the diluted, but unreacted, sample to be used as a correction for carotenoids present.

Reading A is composed of the sum of the absorptions produced by an unknown amount of vitamin A, represented by amount L, and the three types of interfering colored reactions previously mentioned, and represented by quantities M, N, and O, indicating decreasing color, increasing color, and stable color, respectively. Reading B, at the end of 5 minutes, shows that L and M have faded out completely, O has remained constant, and N has increased by increment X. At the end of 10 minutes, represented by reading C, an additional increment, X, has been added to the value of original quantity N, making its value $N + 2X$.

$$\text{Reading } A = L + M + N + O \quad (1)$$
$$\text{Reading } B = X + N + O \quad (2)$$
$$\text{Reading } C = 2X + N + O \quad (3)$$

from which the formula $L = A - 2B + C - M$ may be derived. M may be derived by a separate reading (reading E) of the carotenoid color at 440 mμ which will bear a relationship, K, to the blue color produced at 620 mμ for any particular group of pigments and pair of filters.

Hence, $$M = KE$$

or $$L = A - 2B + C - KE$$

To compensate for the fact that certain materials may inhibit the amount of absorption L produced by the antimony trichloride reaction with vitamin A, a known increment (10 units = S) of A is added to a second fraction of the original solution and after adjusting to the same volume for reaction, reading D is obtained.

All color produced by preformed vitamin A has completely faded at the end of 5 minutes. Thus, after this period of time the sample with the added increment of vitamin A will read the same as the sample without the vitamin A increment (Figure 1). This fact makes it possible to use the principle of the internal standard with its attendant advantages as stated by Oser, Melnick, and Pader (*7*).

$D - A$ equals the amount of absorption produced by 10 units of vitamin A under the same reaction conditions as in the case of L absorption, produced by the unknown amount of vitamin A.

For low concentrations of vitamin A the readings given in terms of photometric density are directly proportional to the amount of vitamin A present.

$$\text{Therefore} \qquad \frac{\text{Units of vitamin A}}{10} = \frac{L}{D - A}$$

$$\text{or} \qquad \text{Units of vitamin A} = \frac{10(A - 2B + C - KE)}{D - A}$$

The value of K is derived by reacting with antimony trichloride and reading at 620 mμ portions of a series of solutions of varied carotenoid concentration and reading dilutions of the same solutions at 440 mμ. The reacted absorption values are plotted against the dilution absorption values and a straight-line relationship is found. The ratio of the photometric density at 620 mμ to that at 440 mμ is the value of K desired. This was found to be 0.067 for the authors' instrument for the carotenoids of alfalfa, the major carotenoid source in feeds. This value was obtained after a chromatographic column treatment and readings obtained according to the procedure given. The chromatographic treatment removes pure carotene and only the related pigments are present.

Table I. Composition of Feed Mixtures

Ingredient	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
	%	%	%	%	%	%
Soybean oil meal	30	30	30	30	30	...
Corn meal	50	50	50	...	...	...
Tankage	20	...	...	...	10	20
Fish meal	...	20	...	20	20	30
Ground oats	...	...	...	30	...	...
Alfalfa	...	...	20	20	20	30
Wheat germ	...	...	...	...	20	20
Total	100	100	100	100	100	100

RECOVERY OF ADDED VITAMIN A

In order to check on the accuracy of the reading procedure, samples of feedstuff mixtures (Table I) were prepared containing varying proportions of typical feed materials. Some of the mixtures were prepared with abnormally large amounts of certain types of ingredients known to interfere with vitamin A measurement.

Portions of these mixtures were extracted and purified on chromatographic columns. Each extract was divided into two equal portions, each equivalent to 8 grams of original sample and one portion was fortified with 100 I.U. of vitamin A or 12.5 I.U. per gram of sample. The two portions of each sample were then treated as separate samples and their vitamin A content was read and calculated by the procedure given. The value of X for calculation is 2.5. According to the procedure later adopted a whole number, in this case 3, is recommended.

Table II shows the results obtained on this experiment. The average recovery obtained, 12.61 I.U. per gram, checks well with a theoretical recovery of 12.5. The range 11.62 to 13.60 is within the range to be expected from a reading procedure depending upon five independent readings.

Table II. Recovery of Added Vitamin A

Sample No.	Reading A	Reading B	Reading C	Reading D	Reading E	Vitamin A Found *I.U./g.*	Recovery *I.U./g.*	Error *I.U./g.*
1	0.027	0.022	0.032	0.214	0.034	0.43	...	....
1F[a]	0.382	0.034	0.043	0.540	0.034	14.03	13.60	+1.10
2	0.032	0.041	0.056	0.214	0.045	0.10	...	....
2F[a]	0.387	0.051	0.062	0.551	0.045	13.10	13.00	+0.50
3	0.149	0.122	0.122	0.328	0.403	0.00	...	....
3F[a]	0.485	0.131	0.131	0.661	0.403	11.62	11.62	−0.88
4	0.161	0.144	0.149	0.340	0.475	−0.35	...	....
4F[a]	0.512	0.158	0.161	0.671	0.475	12.77	13.12	+0.62
5	0.174	0.155	0.172	0.358	0.462	0.17	...	....
5F[a]	0.509	0.164	0.181	0.677	0.462	12.32	12.15	−0.35
6	0.201	0.177	0.197	0.381	0.495	0.38	...	....
6F[a]	0.530	0.184	0.204	0.696	0.495	12.54	12.16	−0.34
					Av.	0.12	...	
						12.73	12.61	

[a] Same as samples 1, 2, etc., except 12.5 I.U. per gram added vitamin A.

None of the ingredients involved was believed to contain preformed vitamin A and an average value for the unfortified samples of 0.12 I.U. per gram with a range of −0.35 to +0.43 is acceptable.

Previous experience in this laboratory has shown that the chromatographic procedure recommended completely separates the carotene from the vitamin A and that the amount of xanthophyll pigments present is reduced to a minimum. As a check on the recovery of vitamin A carried through the whole procedure, two formulas for commercial mixed feed concentrates containing no preformed vitamin A and known to give very bad reaction colors with antimony trichloride were fortified at several vitamin A levels. These samples were carried through the complete analytical procedure and the recovery of vitamin A was measured (Table III).

The unfortified samples gave results of 0.07 and −0.21 I.U. per gram, which is in line with those expected, and assuming a zero value for the unfortified samples the amount of vitamin A lost in the complete procedure varied from 0.74 to 0.88 I.U. of vitamin A per gram of sample. Since this is the combined loss of the extraction, saponification, and column procedure, plus losses in transferring and evaporation, it appears to be satisfactory for this type of procedure. However, this loss is apparently an absolute one and for very low potency feeds it may result in a high percentage error.

No attempt is made in this paper to measure vitamin A activity other than that produced by preformed vitamin A. Carotene can be measured on the first eluate from the chromatographic column but the problems involved in the separation and measurement of different carotenoids having vitamin A activity and the subsequent conversion into units of preformed vitamin A are at present only partially solved.

Table III. Recovery of Added Vitamin A from Mixed Feeds

Sample	Added Vitamin A *I.U./g.*	Total Vitamin A *I.U./g.*	Loss of Vitamin A *I.U./g.*
Feed A	...	0.07	..
Feed A	8.20	7.32	0.88
Feed A	10.24	9.48	0.76
Feed A	16.40	15.52	0.88
Feed B	...	−0.21	..
Feed B	10.24	9.50	0.74

SUMMARY

A modification of the antimony trichloride method for the determination of vitamin A in feeds and feedstuffs compensates for the presence of interfering reacting materials by means of the differential effect of light on the kinetics of the antimony trichloride reaction of vitamin A and the interfering materials, respectively. A chromatographic procedure suitable for the preparation of feed extracts intended for color reaction is given. The principles of the procedure appear favorable for use in a variety of applications in the determination of vitamin A in human foods.

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Direct Volumetric Determination of the Organic Sulfonate Content of Synthetic Detergents

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A method has been developed for the quantitative determination of sodium alkylbenzene sulfonates, which is applicable to commercial detergents containing alkyl or alkylaryl sulfonates. Under standardized conditions *p*-toluidine hydrochloride reacts with these organic sulfonates to give amine-sulfonate salts which can be subjected to rapid and relatively precise quantitative analytical determination suited to the requirements of industrial laboratories.

THE usual methods for obtaining the active organic content of a detergent mixture are relatively long and produce results calculated by "difference" methods (*2*, *4*). In a search for a rapid and direct mode of analysis for production control to overcome the time disadvantages of these methods a procedure has been developed which has considerable merit for the analysis of certain detergents of either the alkyl sulfonate or alkylaryl sulfonate type. ("Alkyl" is used throughout to indicate hydrocarbon species obtained by fractional distillation of petroleum.) The new method employs the familiar qualitative reaction of amines and aryl sulfonic acids (*5*, *7*) with modifications to obtain quantitative results of relatively high precision and accuracy under standardized conditions.

METHOD FOR ALKYLBENZENE SULFONATES

A neutral detergent mixture containing the sodium salts of alkylbenzene sulfonic acids as the active organic ingredient may be reacted in aqueous medium with an amine salt of a strong inorganic acid to produce the sulfonic acid salt of the amine and the sodium salt of the inorganic acid. Removal of the sulfonate-amine complex into a water-immiscible phase will displace the equilibrium to favor completion of the reaction. The homologous nature of the organic species in most commercial detergents facilitates this removal by prevention of crystallization. The organic sulfonate can then be determined by suitable treatment of the solvent extract of the reaction mixture. Direct titration with standard alkali in the presence of a suitable indicator has been found rapid and convenient. The weakly basic amine does not interfere. The main reactions may be presented as follows:

$$RC_6H_4SO_3NA + CH_3C_6H_4NH_2.HCl \longrightarrow CH_3C_6H_4NH_2.RC_6H_4SO_3H + NaCl$$

$$CH_3C_6H_4NH_2.RC_6H_4SO_3H + NaOH \longrightarrow RC_6H_4SO_3Na + CH_3C_6H_4NH_2 + H_2O$$

After numerous trials with various amine salts and solvents, *p*-toluidine hydrochloride and carbon tetrachloride were selected for greatest ease of manipulation. The following specific procedure was devised for application to solid detergents containing 30 to 60% sodium alkylbenzene sulfonate and remainder of neutral inorganic salts.

A weighed sample containing 3 to 4 grams of the organic material is transferred to a 250-ml. (Corning No. 6340) separatory funnel (stem cut to 1.25 cm., 0.5 inch). Fifty milliliters of C.P. carbon tetrachloride and 100.0 ml. of an aqueous solution containing 3.40 grams of *p*-toluidine hydrochloride (*1*) are added; the funnel is then stoppered and shaken mechanically at room temperature for about 5 to 10 minutes or until all the solid phase has disappeared. Complete layer separation is effected by a few minutes of settling. The carbon tetrachloride layer is carefully drawn off, and the aqueous phase is extracted a second time using a brief shaking with an additional 25 ml. of carbon tetrachloride. The carbon tetrachloride extracts are combined in 100 ml. of 95% ethanol previously just neutralized with 0.1 *N* sodium hydroxide to a faint purple end point using *m*-cresol purple as the internal indicator. The resulting solution is titrated in a glass-stoppered, 500-ml. Erlenmeyer flask with 0.1 *N* sodium hydroxide until the emulsion obtained by vigorous intermittent shaking during titration tends to remain a lavender color. (Alcohol is used for facilitating the titration reaction and discerning the end point; formulas 2B and 3A are satisfactory.)

The organic content of the sample is calculated from the "% organic" equivalent of the standard sodium hydroxide solution found by carrying out the above determination on a suitable reference sample of known organic content.

SAMPLE CALCULATION. A reference sample was found to have an organic content of 40.0% when analyzed by "difference" [100% − (% sodium sulfate + % moisture + % sodium chloride + % ether-soluble)]; it required 67.32 ml. of 0.10 *N* sodium hydroxide per 6.000-gram sample by the procedure described; therefore, the factor for converting ml. of 0.10 *N* sodium hydroxide to per cent of organic material in unknown samples of the same organic constitution $= \frac{40.0}{67.32} = 0.594$ per 6 grams of sample.

A 6.000-gram sample of an unknown required 92.00 ml. of 0.10 *N* sodium hydroxide; hence its organic content = ml. of NaOH × 0.594 = 54.6%.

In order not to confine narrowly the apparent applicability of the method, calculations on a stoichiometric basis are avoided in the general procedure. Because the weight of the average of the alkyl groups is not always definitely known or is subject to variation with modifications of manufacturing processes and because of the empirics of reagent volumes in this method, some products may not be accurately analyzed when calculated factors are employed.

DISCUSSION OF VARIABLES

When the initial concentration of *p*-toluidine hydrochloride is fixed for a series of determinations in which only the weight of sample is varied, the alkali equivalent per gram of sample remains constant when the molecular ratio of amine to sulfonate exceeds about 2.5.

When the outlined analytical procedure is applied to a series of duplicate samples, except that the *p*-toluidine hydrochloride concentration in the amine reagent is varied, it appears that the alkali equivalent per gram of sample is regularly affected by the initial concentration of the amine hydrochloride. Data from such a series are plotted in Figure 1. Physical limitations fix the range of reagent concentration. The amine salt in concentrations above about 8% on a weight-volume basis causes phase separation difficulties; when its initial concentration is much below 2.5 times the molar concentration of the sulfonate and below about 3.4 grams per 100 ml. of reagent, sufficient detergent remains unreacted to cause emulsification. It is not known why the curve of Figure 1 does not become parallel with the abscissa at the point demanded by theory, but since a continuous line function is exhibited over a wide range of amine concentrations and since chlorides can be qualitatively detected in the carbon tetrachloride extract, it is likely that, although *p*-toluidine hydrochloride is in-

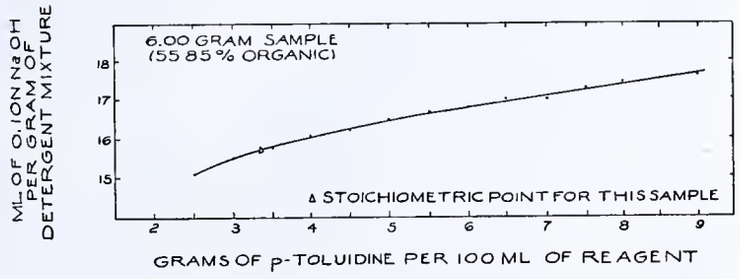

Figure 1. Variation of Alkali Titration per Gram of Sample with Initial Concentration of *p*-Toluidine Hydrochloride

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Table I. Analysis of Commercial Products by *p*-Toluidine Hydrochloride Method

Product	Type of Molecule (*3*, *6*, *8*)	Ml. of 0.10 *N* NaOH per Gram
Santomerse 1	Alkylaryl sulfonate	10.3
Santomerse 3	Alkylbenzene sulfonate	26.2
Santomerse D	Alkylbenzene sulfonate	26.8
MP 189	Alkyl sulfonate	9.7
SV 1959	Alkyl sulfonate	23.8
Ultrawet A	Alkyl sulfonate	28.7
Nacconol NR	Alkylbenzene sulfonate	11.1
Nacconol HG	Alkylbenzene sulfonate	15.2
Nacconol F	Alkylbenzene sulfonate	12.8
Naccolene F	Alkylbenzene sulfonate in solvent	2.7
Naccosol A	Alkylnaphthalene sulfonate	26.7

soluble in carbon tetrachloride, it is taken into the solvent layer in slight amounts by small changes in miscibility related to an effect of *p*-toluidine hydrochloride concentration.

However, by strict adherence to volume and reagent concentration in a procedure with any specified concentration of amine hydrochloride in the graphed range, one may obtain reproducible results for alkali equivalent per gram of sample when the weight of sample is varied within its practical limits. Notwithstanding, the reagent concentrations at which there apparently is stoichiometric relationship have been selected for the authors' use to avoid occult errors. These concentrations were determined from application of the method to mixtures of commercial detergent with pure sodium dodecylbenzene sulfonate, the former being employed in admixture to prevent formation of a crystalline product by the latter.

When the stoichiometric point is found by use of a suitable pure compound of known molecular weight, the average molecular weight of the mixture of homologs in a commercial detergent can be found; hence, conditions at which the metathetical amount of alkali is used at the titration end point can then be determined for other detergent mixtures for which a standard factor may be desired to convert titrations to per cent organic content.

The data in Figure 1 illustrate those obtained on portions of a detergent sample for which the average molecular weight had been calculated. The point of apparent stoichiometric relationship chosen for fixing reagent quantities and volume was selected by use of this calculation.

For only routine or occasional analytical purposes it is not necessary to determine the optimum amine salt concentration; if a desired reference sample is run with the unknown under identical conditions of volume and amount of reagents, the concentration may be between 3 and 8%.

Perchloroethyle ne may readily be used to replace the carbon tetrachloride, but other solvent substitutions should be made only after an adequate study of the effects involved.

In the presence of colored materials which are otherwise negligible in effect, a pH meter and glass electrode may be used to determine the titration end point in an open vessel if vigorous stirring is provided.

RESULTS

Determinations by five analysts using fifty-seven samples of detergent of known organic content (35 to 55%) established that the *p*-toluidine method as outlined above is precise to ±0.23%. The accuracy is subject to sampling errors and to accuracy of the standardization of alkali in terms of organic titer. In routine practice, however, over a 12-month period, results by this method have checked those obtained by the usual "difference" methods to ±0.5%, and have cut the elapsed time for a single determination from about 5 hours to 20 minutes.

By slight variations in technique the method proposed for solid detergent mixtures has been applied successfully to various process slurries and to liquid detergent and wetting preparations containing nonreactive diluents. Replacement of benzene with toluene, phenol, or naphthalene as the source of aryl group in the detergent introduced no complexity of manipulation.

Sodium benzene sulfonate alone or mixed with detergent samples remained undetected under the conditions of the method.

A few of the commercial detergents tested for adaptability to the outlined procedure are listed in Table I. Data were not available on all the samples for drawing inferences about accuracy from the titration values, so such calculations were omitted entirely. Each sample seemed to have sufficient homologous nature to make it amenable to this procedure without the undesirable crystallizing effects exhibited by such compounds as pure dodecylbenzene sodium sulfonate.

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New Standard Samples

The National Bureau of Standards, Washington 25, D. C., has inaugurated a series of standard samples for use in the preparation of buffer solutions of known pH values from 0° to 60° C., and in the calibration of instruments for the measurement and control of pH. Three standards of this series are now available, acid potassium phthalate, potassium dihydrogen phosphate–disodium hydrogen phosphate, and borax (sodium tetraborate decahydrate). The two phosphates are intended to be used together. Four new standard steels have also been added to the list. Table I shows sample numbers and fees of the new standards.

Orders should give both the number and the name of the sample wanted. No samples of smaller size than those listed are distributed, and remittance should accompany order.

The bureau now issues more than 300 different kinds of standard samples, comprising steels, irons, ferroalloys, nonferrous alloys, ores, ceramic matcrials, certain high-purity chemicals, hydrocarbons, paint pigments for color, oils for viscometer calibrations, certain reference standards, and melting-point standards. Complete information is given in the Supplement to Circular C398, which can be obtained free of charge upon application to the bureau.

Table I

Sample No.	Material	Recommended Concentration *Mole/liter of solution*	pH Value at 25° C.	Approximate Weight of Sample *Grams*	Price per Sample
185	Acid potassium phthalate	0.05	4.005	60	$3.00
186	Potassium dihydrogen phosphate (186-I)	0.02	6.866	60	6.00
	Disodium hydrogen phosphate (186-II)	0.02			
187	Borax	0.01	9.177	30	3.00
139	Steel (N.E. 8637) (approximately 0.5 Ni, 0.5 Cr, 0.17 Mo)			150	3.00
152	Steel (B.O.H.) (tin-bearing approximately 0.04 Sn)			150	2.00
155	Steel (approximately 0.5 Cr, 0.5 W)			150	3.00
156	Steel (N.E. 9450) (approximately 1.4 Mn, 0.5 Ni, 0.4 Cr, 0.13 Mo)			150	3.00

Colorimetric Determination of Nickel in Bronzes

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A method is described for the colorimetric determination of nickel in bronzes. Copper is separated by precipitation with ammonium hypophosphite and subsequent filtration. The nickel is then determined by measurement of the color produced by dimethylglyoxime in the presence of citric acid, iodine, and ammonia. The method is rapid (one determination can be completed in less than 30 minutes) and of sufficient accuracy for routine purposes.

VARIOUS color reactions have been suggested for the colorimetric determination of nickel but dimethylglyoxime is so far still the best reagent for this particular purpose. This reaction is based on the fact that a red or reddish-brown solution is produced when dimethylglyoxime is added to an alkaline solution containing nickel, provided that it has previously been treated with an oxidizing agent such as bromine water or iodine solution. The colored compound is nickelic (IV) dimethylglyoxime. Its color depends on the method of preparation—i.e., whether the oxidant was added to the solution when it was still acid or after it had been made alkaline.

Feigl (*3*) first described this reaction and Rollet (*14*) suggested a few modifications in order to make it suitable for colorimetric purposes. Subsequent investigators have used it for the determination of nickel in steel (*2*, *5–12*, *14*, *16*), where its use has become extensive. The interference of iron can easily be eliminated by adding citric or tartaric acid. However, the method is not directly applicable in the presence of considerable amounts of copper, and as far as the present authors can ascertain, there have been only three methods described in the literature for its application to the determination of nickel in bronzes (*1*, *4*, *13*).

Haywood and Wood (*4*) recommend the direct colorimetric determination of nickel in the presence of copper. By the use of special filters and a suitable blank the difficulties arising from the presence of copper are overcome, but this method can be used only for nickel contents up to 5%.

There is an earlier reference by Dietrich (*1*) to a similar method, but apart from stating that nickel is determined in an aliquot portion of the solution used for copper, the only information given is that bromine water, ammonia, and dimethylglyoxime are required. Results could be obtained within 10 to 15 minutes for nickel contents up to 15%.

Ochotin and Sytschoff (*13*) developed a method for the colorimetric determination of nickel in alloys, which they claim to be rapid and accurate enough for routine analysis. But they state that if copper is the major constituent of such an alloy a separation—e.g., by electrolysis—has to be carried out although this separation need not be quantitative. After removal of the copper the nickel is precipitated by dimethylglyoxime. The solution containing the precipitate is then transferred to a separating funnel and treated with ether, with the effect that the entire precipitate becomes dispersed in the ether. The aqueous solution is then drained off and alcohol and collodion are added to the ether suspension. The resulting colored solution is compared with standard solutions of known nickel content prepared under similar conditions. This method was tried out but the separation with ether was tedious and investigations to find a more practical method were continued.

Since the method for steel was not directly applicable to bronzes on account of the interference of the copper, the problem was to find a quick method of separating this element. As small quantities of copper are not detrimental, such a separation need not necessarily be quantitative. The usual methods of separating copper—i.e., by electrolysis or by gassing with hydrogen sulfide—were not adopted, as the former requires the use of additional apparatus and the latter is objectionable on account of the obnoxious fumes of the hydrogen sulfide, and the tendency of copper sulfide to become colloidal makes it difficult to filter. A search through the literature was, therefore, carried out to find other suitable methods of separation. It was found that Turbin (*15*) had attempted to separate copper and nickel by means of iron powder. This was tried out but results were not very promising. Next, an attempt was made to reduce the copper by means of a sugar solution in the presence of alkaline sodium potassium tartrate. This reaction is commonly used for the determination of sugar in urine and it was considered that it might well be applied in the reverse direction. The tests were fairly successful and the only disadvantage appeared to be caramelization of the sugar which made it necessary to boil the solution afterwards with nitric acid in order to destroy the color.

Finally, another method was investigated using a solution of ammonium hypophosphite as reducing agent. This effectively precipitates the copper at low acid concentrations, yielding a precipitate which is easy to filter. Although the copper is not quantitatively removed in this way, the small amount which remains does not seriously affect the subsequent colorimetric determination of nickel. An excess of hypophosphite has no influence on the reaction, as sufficient iodine is added for its complete oxidation.

REAGENTS USED

Ammonium hydroxide, specific gravity 0.88.
Ammonium hypophosphite solution, 5%.
Citric acid solution, 10%.
Dimethylglyoxime solution, 1% in alcohol.
Iodine solution, 0.1 *N*: 12.69 grams in 50 ml. of water containing 25 grams of potassium iodide. Solution made up to 1000 ml.
Nitric acid, 1 to 1.

PROCEDURE

Transfer 0.1 gram of fine drillings of the alloy to a 100-ml. beaker and add 2 ml. of nitric acid. Heat until solution is complete and then evaporate gently until a greenish color is obtained. Dilute to 20 ml. with distilled water and add 5 ml. of ammonium hypophosphite solution. Heat to boiling and boil for 3 minutes, then remove from hot plate and allow to cool. Filter through a Whatman No. 541 filter paper (11-cm.) and wash with tepid water until bulk is approximately 150 ml. Cool the filtrate, transfer to a 250-ml. volumetric flask, and make up to the mark. Transfer 25 ml. of this solution to a 100-ml. volumetric flask and add in the following order, shaking well after each addition:

10 ml. of citric acid
2 ml. of iodine solution
10 ml. of ammonium hydroxide solution
4 ml. of dimethylglyoxime solution

Finally make up to 100 ml. with distilled water.

Shake the flask well and allow to stand for at least 10 but not more than 30 minutes, and measure the color produced by means of a Zeiss Pulfrich photometer, using the photometer filament lamp with filter S53 (having a mean transmission of 5300 Å.), and distilled water as comparison. (Any other type of colorimeter may be used with suitable filters.)

The method as described above was found to be applicable for nickel contents up to 5%. For nickel contents between 5 and 10% a 10-ml. aliquot should be taken and the result multiplied by 2.5. For nickel contents above 10%, proportionately smaller aliquots should be taken.

This method was first investigated on standard solutions containing a known amount of nickel and copper (Table I). Solution A contained 10 grams of copper per liter and solution B contained 0.2 gram of nickel per liter. These solutions were mixed in the proportions indicated and treated in the way described above.

The graph based on these figures was found to be a straight line passing through the origin, proving that the color is strictly proportional to the concentration of nickel, and thus truly follows Beer's law.

Table I. Determination of Nickel in Standard Solutions

Solution	Ni, Mg.	Cell, Mm.	Reading	K
1. 10 ml. A + 5 ml. B	1.0	30	0.36	0.12
2. 10 ml. A + 10 ml. B	2.0	30	0.76	0.253
3. 10 ml. A + 15 ml. B	3.0	10	0.36	0.36
4. 10 ml. A + 20 ml. B	4.0	10	0.49	0.49
5. 10 ml. A + 25 ml. B	5.0	10	0.63	0.63
6. 10 ml. A + 30 ml. B[a]	6.0	20	0.58	0.725

[a] 10-ml. aliquot taken and reading multiplied by 2.5; hence: Adjusted coefficient = 0.725

Table II. Determination of Nickel in Standard Bronze

Standard	Cell	Reading		Ni (from Standard Graph)	Ni (Gravimetric)	Deviation
	Mm.		*K*	%	%	%
HAR	30	0.21	0.07	0.55	0.50	+0.05
HAS	30	0.99	0.33	2.67	2.66	+0.01
HAT	30	1.00	0.33	6.75	6.72	+0.03
HAU	10	0.40	0.40	7.98	7.92	+0.06
DTD 174	30	0.50	0.167	1.37	1.35	+0.02
DTD 164	30	0.02	0.34	2.76	2.75	+0.01
DTD 197	10	0.63	0.63	5.13	5.10	+0.03
DTD 412	10	0.60	0.60	4.90	4.88	+0.02
DTD 498	30	0.84	0.28	2.27	2.22	+0.05
DTD 504	30	0.78	0.26	2.10	2.05	+0.05
P-bronze	30	1.22	0.407	3.30	3.28	+0.02
Mn-bronze	30	0.50	0.167	1.34	1.14	+0.20
Ni-bronze	30	1.30	0.433	17.6	17.5	+0.10

The method was then tried out for a number of standard bronzes, the nickel content of which had previously been obtained gravimetrically (Table II).

In the case of the high-nickel bronze, the solution was made up to 500 ml. and a 10-ml. aliquot taken. The reading was then multiplied by 5. The results corresponded well with the gravimetric values, the greatest accuracy being found for nickel contents above 3%.

The time required for a single determination is approximately 30 minutes, and very much less if a batch of samples is investigated at the same time.

ACKNOWLEDGMENT

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Removal of Peroxides from Organic Solvents

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A rapid and efficient method for removal of peroxides from organic solvents by means of activated alumina is presented. Since no moisture is added, the method is directly applicable to dioxane and anhydrous solvents.

THE autoxidation of ethers and certain other organic solvents during storage gives rise to the formation of peroxides. The distillation of any of a wide variety of ethers containing appreciable quantities of peroxide may result in dangerous explosions (*14*). Peroxide-containing isopropyl ether appears to be particularly hazardous in this respect, violent explosions of its distillation residues having occurred as a result of heating at temperatures well below 100° C. or even from mechanical shock alone (*3*, *7*).

The octane numbers of synthetic gasolines are appreciably lowered by the formation of peroxides during storage, but can be largely restored by removal of the peroxides (*1*, *11*).

The use of peroxide-containing liquids as solvents for substances which are easily oxidized necessitates the prior removal of the accumulated peroxides. A large number of methods for accomplishing such purifications have been proposed from time to time.

Peroxides, aldehydes, unsaturated compounds, and acids can be effectively removed from impure ether by shaking with aqueous silver hydroxide precipitated *in situ* with an excess of alkali (*12*). A commonly used laboratory method of freeing ethyl ether of peroxides consists in treating the ether with an aqueous solution of ferrous sulfate (*14*). Aqueous solutions of sodium bisulfite, acidified potassium iodide, sodium sulfite (*14*), and potassium permanganate (*7*) have also been recommended. Peroxides may also be eliminated from ether by distilling over either alkaline pyrogallol or alkaline permanganate and then passing a fine spray of the condensed ether through a strongly alkaline solution of either reagent (*8*). All these methods have the disadvantage of necessitating the addition of water which, if an anhydrous solvent is desired, must subsequently be removed. Furthermore, they are not suitable for the treatment of many water-miscible liquids such as dioxane or some of the monoalkyl ethylene glycol ethers (Cellosolves).

The purification of ether with alkali metals (*10*) or hydroxides (*6*, *10*) eliminates both peroxides and aldehydes but, in common with the above methods, also requires a distillation procedure. Two recently developed methods for destroying peroxides are applicable to dioxane as well as to some of the acyclic ethers (*3*). These consist in shaking the dioxane with stannous chloride and distilling off the dioxane and refluxing with lead dioxide and filtering through a tight filter paper.

The only previous report on the use of an adsorbent for the elimination of peroxides in ether appears to be that of Rae (*9*) who found that when peroxide-containing ether was shaken with 1.1% of animal charcoal and allowed to stand, the peroxides gradually decreased and in 54 days finally disappeared. Harris and Welch (*4*) found that certain carbons removed the compounds which were responsible for a positive Kreis test in a cottonseed oil.

Activated alumina has previously been recommended for the continuous commercial drying of organic liquids (*2*). In connection with the chemical study of certain oxygen-labile steroids in these laboratories, it was found that peroxides could be completely and quickly removed from many organic solvents by merely passing them through a vertical column of activated

alumina. The peroxides were not decomposed or converted into other products by the alumina, but were removed from solution by adsorption. The aldehyde contents were also reduced by this treatment, as was evidenced by testing with 2,4-dinitrophenylhydrazine. The purified solvents did, however, give positive reactions with the Schiff fuchsin-aldehyde reagent. Because the urgency of other problems prevents undertaking a more comprehensive study of the removal of peroxides and aldehydes by adsorbents and because of the potential usefulness of this technique to others, the results are being published at this time.

PROCEDURE

Grade F-20 chromatographic Alorco activated alumina (−80-mesh) was supported in an upright chromatographic adsorption tube by a clean cotton plug. The dry alumina was poured into the tube while the tube was being jarred to obtain good settling of the adsorbent. A wisp of cotton placed on top of the adsorbent protected the surface of the adsorbent from agitation.

A generous layer of the solvent to be purified was placed on top of the adsorbent, the remainder of the solvent then being added by means of a dropping funnel fitted into the top of the tube with a rubber stopper. The flow of the liquid through the column was greatly accelerated by means of air pressure applied to the liquid in the dropping funnel. The compressed air which furnished the air pressure was passed through a safety bottle of the type commonly used in reduced pressure distillations. The valve of the safety bottle was left open. This prevented the building up of objectionable pressures. The pressure was regulated satisfactorily by adjustment of the valve in the compressed air line.

Determination of Peroxides. Peroxide values of the original solvents as well as of successive portions of solvents which had passed through the alumina columns were determined by an iodometric method based on Wheeler's method of determining peroxides in fats and oils (*13*). Five to 50 ml. of solvent, depending upon its peroxide content, were mixed with 30 ml. of a 40 to 60 chloroform–glacial acetic acid mixture. One milliliter of saturated aqueous potassium iodide solution was added and the flask was rotated for 1 minute. At the end of the 1-minute interval, 50 ml. of water were added and the mixture was immediately titrated with 0.01 *N* sodium thiosulfate, using starch as an indicator.

Table I. Comparison of Iodometric and Permanganate Methods for Determination of Peroxides in Solvents

Solvent	Peroxide Content I Iodometric method	Peroxide Content II Permanganate method	II/I
	Micromoles of O_2 per liter		
Aqueous H_2O_2 1	10,000	10,600	1.06
Aqueous H_2O_2 2	333,000	349,100	1.05
Aqueous H_2O_2 2 + formaldehyde[a]	304,400	294,600	0.97
Aqueous H_2O_2 2 + acetaldehyde[a]	155,700	338,200	2.2
Ethyl ether 1	0.0	0.0	...
Ethyl ether 1 + formaldehyde[a]	0.0	0.0	...
Ethyl ether 1 + acetaldehyde[a]	0.0	0.0	...
Ethyl ether 1 + H_2O_2	6,200	9,200	1.5
Ethyl ether 9[b]	4,600	7,850	1.7
Ethyl ether 9 + formaldehyde[a]	4,020	8,510	2.1
Ethyl ether 9 + acetaldehyde[a]	3,840	9,160	2.4
Ethyl ether 2	6,700	9,200	1.4
Ethyl ether 2 + cinnamaldehyde[a]	6,800	138,600	20.4
Ethyl ether 3	127	130	1.02
Ethyl ether 3 through Al_2O_3	0.0	0.0	...
Ethyl ether 4	56,100	63,800	1.1
Ethyl ether 5	46,800	88,100	1.9
Ethyl ether 5 through Al_2O_3	0.0	0.0	...
Skellysolve F	0.0	0.0	...
Dioxane	95,600	36,600	0.4
n-Butyl ether	56,200	12,400	0.2

[a] Solvent allowed to stand 30 minutes after addition of aldehyde before being titrated.
[b] Ether prepared by adding some ether having a very high peroxide content to peroxide-free ether.

Omitting the chloroform–acetic acid mixture and supplying the necessary acid by the addition of 1 ml. of glacial acetic acid did not yield good results—i.e., in some cases (*n*-butyl ether) no satisfactory end point could be obtained and in some cases where a very sharp end point was obtainable (dioxane) the values were low.

The determination of adsorbed peroxide was made on the alumina after its removal from the tube. The peroxides were eluted by shaking the alumina briefly with sufficient chloroform–acetic acid mixture (30 ml. for 18.5 grams of alumina). One milliliter of saturated potassium iodide solution was added and the determination was carried out by the procedure used in determining the peroxides in solvents.

Table II. Removal of Peroxides from Ethers

[1.9 × 33 cm. Al_2O_3 columns (82 grams of Al_2O_3)]

Solvent	Peroxide Content	Volume Completely Freed from Peroxide	Peroxide Removed Per column	Peroxide Removed Per 100 gm. Al_2O_3
	Micromoles O_2/l.	*Ml.*	*Micromoles O_2*	*Micromoles O_2*
Ethyl ether 3 (absolute)[a]	127	700[a]	88.9[a]	...[a]
Ethyl ether 6[b]	1,870	200	374	460
Ethyl ether 7[c]	3,670	250	918	1,120
Ethyl ether 8[d]	31,490	100	3,150	3,840
n-Butyl ether[e]	61,930	350	21,680	26,400
Dioxane 1[f]	37,500	200	7,500	9,150
Dioxane 2[g]	299,600	100	29,960	36,500

[a] Total supply 700 ml. Al_2O_3 column was still able to remove peroxide completely when all available solvent had been treated.
[b] U.S.P. ether in clear glass bottle several months.
[c] U.S.P. ether in clear glass bottle, then dried with $CaCl_2$.
[d] J. T. Baker ethyl ether, "for fat extraction, purified, dried", in a partly filled amber glass bottle in dark for over 3.5 years.
[e] In amber glass bottle over 3.5 years.
[f] Contained no peroxides when received. Opened to admit air, closed, shaken, and allowed to stand in original amber glass bottle for one month.
[g] In screw-top metal container for over 2.5 years.

The iodometric method of determining peroxides in solvents was compared with the permanganate titration method of King (*5*). Table I shows that the permanganate method gave consistently higher results with ethyl ether but that the ratio of these results to those obtained by the iodometric method varied widely for different samples of ether. These differences in results are thought to be due partly to the titration of substances other than peroxide by the potassium permanganate and partly to a possible incomplete recovery of peroxide by the iodometric method. The potassium permanganate titrations for peroxide in dioxane and *n*-butyl ether unexpectedly gave much lower results than the iodometric method, possibly because of a greater stability of these peroxides toward potassium permanganate. All solvents which were shown to be free of peroxides by one method were negative by both methods.

The authors have made no attempt to determine the reasons for the discordant results obtained by the two methods. Because the iodometric method was more convenient and was satisfactory for their purposes, the results reported below were all obtained by this method.

RESULTS

A number of ethers were passed through 1.9 × 33 cm. columns of activated alumina containing about 82 grams of the adsorbent. Peroxide determinations were made on successive 50-ml. portions of each solvent after it had passed through the alumina. Table II shows the volumes of these solvents which were completely freed from peroxides by passage through such a column.

On continuing to pass the solvents indicated in Table II other than ether 3 through the columns, succeeding portions of each solvent contained increasing amounts of peroxide—i.e., the volumes indicated in the table exhausted the ability of the alumina to remove peroxides completely from the respective solvents. Larger columns can, of course, be used to free proportionately larger volumes of solvent from peroxides.

The 700 ml. given in the table for ethyl ether 3 also represent the total supply of this ether which was at hand. It is likely that a very much larger volume of this solvent could have been purified had it been available.

A comparison of ethers 6 and 7 indicates that the moisture content of the ether affects the efficiency of the peroxide removal.

A larger volume of the dried ether was purified even though its peroxide content was double that of the other.

Activated alumina appears to vary in its efficiency for removing peroxides with the nature of the solvent being purified. Thus, its capacity for removing peroxides was much greater for *n*-butyl ether than for ethyl ether.

Activated alumina seemed to work more efficiently with the solvents having the higher peroxide contents. Other factors such as aldehyde or acid content may, however, have been partly responsible for this. The effect of the rate of flow of the solvent was not investigated.

Three Skellysolve petroleum fractions, which contained peroxides, were freed from peroxides by passing each through 18.5 grams of activated alumina in separate columns 1.5 × 10 cm. in size. The peroxides had developed in the petroleum fractions by autoxidation after being redistilled and stored in partly filled clear glass containers exposed to daylight for several months. The volumes which were treated are indicated in Table III. Because these volumes exhausted the available supply of peroxide-containing Skellysolves, the total capacity of the columns for completely removing peroxides from these solvents could not be determined; in no case was the peroxide-removing capacity of the column exhausted.

Table III. Removal of Peroxides from Petroleum Fractions

[1.5 × 10 cm. Al_2O_3 columns (18.5 grams of Al_2O_3)]

Petroleum Fraction	Peroxide Content	Volume Treated and Completely Freed of Peroxide	Peroxide Removed
	Micromoles O_2/l.	*Ml.*	*Micromoles O_2*
Skellysolve B	154.0	648[a]	99.8
Skellysolve C	33.6	600[a]	20.2
Skellysolve F	48.0	650[a]	31.2

[a] These volumes exhausted supply of solvents. Much larger volumes probably could have been purified had they been available.

That activated alumina removes peroxides from solvents by adsorption rather than by decomposition is clearly indicated in Table IV. The "peroxide removed from solvent" was calculated from the difference in the peroxide content of the solvent before and after passing through the alumina column. The "adsorbed peroxide" was determined by titrating the alumina itself after passage of the organic liquid.

The removal of peroxides from organic solvents by means of activated alumina appears to have certain advantages over most of the methods which have been reported. The fact that the elimination of peroxides by alumina takes place by adsorption is of decided advantage in purifying solvents in those instances where the presence of peroxide decomposition products might be objectionable. The method is simple and rapid and requires no equipment not readily available. Since no moisture is added to the solvent to be purified, the method is directly applicable to dioxane and to anhydrous solvents.

Table IV. Adsorption of Peroxides

(18.5 grams of Al_2O_3)

Solvent	Peroxide Removed from Solvent	Adsorbed Peroxide Found in Alumina by Titration
	Micromoles O_2	*Micromoles O_2*
Ethyl ether	87.4	86.4
Methyl Cellosolve	34.6	35.4
Skellysolve B	99.8	96.0
Dioxane	2,990.0	3,040.0

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A Constant Reflux Ratio Distilling Head

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ONE of the most troublesome problems in carrying out a distillation of laboratory or pilot-plant scale is the maintenance of a constant reflux ratio. In any distillation upon which calculations are to be made and on many precise analytical distillations as well, it is essential that the reflux ratio be constant and known.

The simplest and most common way of obtaining reflux ratio is use of a stopcock to remove a fraction of the liquid from the total condensate. Partial constriction of a capillary with mercury (*6*) or a nonlubricated glass value (*8*, *10*) has been used to eliminate the stopcock (*6*). Some shortcomings and operating difficulties associated with still heads which depend upon throttling of product for reflux control are (*4*):

1. The reflux must be laboriously and painstakingly controlled by manual adjustment of the stopcock.
2. The reflux ratio must be determined by drop-counting—a time-consuming and often inaccurate process.
3. Accumulation of dirt particles, grease, or drops of insoluble, high surface tension liquid such as water in the regulating stopcock greatly affects the reflux ratio, usually requiring interruption of the distillation.
4. The reflux ratio varies with the rate of distillation, since the rate of product take-off remains fixed.
5. A heterogeneous (two-phase) system cannot be distilled because more of one layer, usually the lower, is removed and equilibrium is thus destroyed.

The accumulative effects of these difficulties result in frequent, sometimes almost constant, attention to each still in order to get satisfactory distillation; uncertainty and variability of the conventional still head, making it difficult to do a reproducible and good analytical distillation; near impossibility of carrying out a

distillation suitable for precise calculation; and inability to handle a distillation yielding a heterogeneous overhead product.

Throttling the vapor instead of the condensed product eliminates the difficulty encountered with heterogeneous distillates.

A constant reflux ratio has been obtained by the use of intermittent take-off, by dividing the condensate into two streams, or by dividing the condensing surface into proportional parts. The intermittent take-off type usually employs a solenoid operated by a timing device. The removal of a portion of the condensate is effected by a swinging funnel (*2*), a swinging wire, a ball and seat, or a plunger and seat (*7*). One of the latest intermittent arrangements utilizes pressure change to withdraw the product (*9*). Most of these devices are not easily built and in addition have the difficulties associated with a complex system and many moving parts.

Dividing the condensed phase into two streams has been accomplished by capillaries (*1*) or by a glass arc (*5*).

Heads separating reflux by means of proportional condensing surfaces are of two general types. One type consists of a number of vertical tubes in parallel arranged similar to a tube bundle heat exchanger. The vapor is condensed on the inner surface of the tubes. Funnels are placed under a few of the tubes to catch the distillate, the condensate from the remaining tubes being returned as reflux. The Corad type head (*3*) condenses the vapor on the inside of one tube which is divided by means of vertical strips into several parallel surfaces. The condensate from one surface is taken off as product while the remainder is returned as reflux to the column. These heads operate very satisfactorily, but they are not easily fabricated from glass.

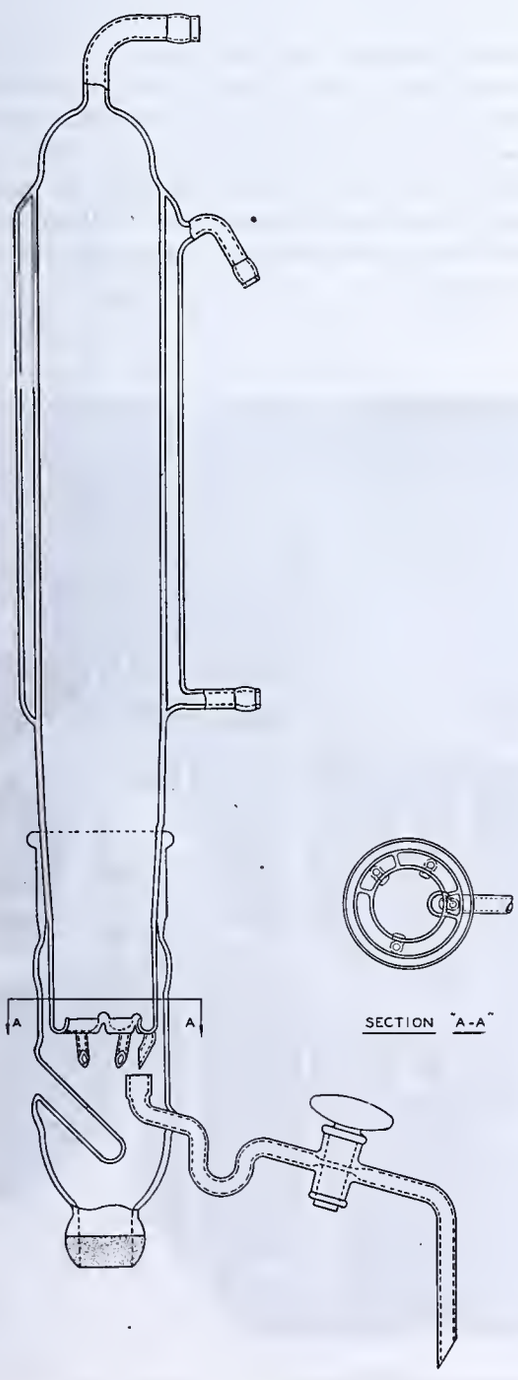

Figure 1. Distilling Head

A distilling head of the nonintermittent type has been designed (Figure 1) which gives a constant reflux ratio regardless of distillation rate, has no moving parts, and can be readily built by a skilled glass blower. The vapor is condensed on the concave (inside) surface of a vertical tube which has a gutter or trough around its lower inside perimeter. The trough is divided into sections and each section has a hole and its own downspout or drip. The downspouts are arranged to lie in a circle and either they or the receiving cup may be rotated to take product from any (or none) of the downspouts. The length of the section of trough compared with the total trough length determines the reflux ratio obtained from each section.

Figure 1 shows the arrangement when the receiving cup is fixed and the downspouts are rotatable by means of a standard-taper ground-glass joint. The head shown had the condensing section made from 48-mm. tubing, the lower chamber from 56-mm. tubing; the downspouts, 5-mm. tubing; a 55/50 standard-taper joint to rotate the downspouts, and a 35/25 ground-glass ball joint to attach it to the distilling column. The trough was divided into four sections, approximately 180, 90, 60, and 30 degrees. This would give reflux ratios (amount returned: amount removed) of 1 to 1, 3 to 1, 5 to 1, and 11 to 1. Calibration of the head gave actual reflux ratios of 0.9 to 1, 3.8 to 1, 5.2 to 1, and 8.5 to 1. The only critical dimension seems to be the diameter of the downspout giving the lowest reflux ratio. It must be sufficient to prevent trough overflow up to column capacity. The condensing tube must be clean and smooth and be kept vertical, so that channeling will not occur. The head operates satisfactorily under vacuum, provided the receiver is connected by an additional line to the vent at the top of the condenser. Heterogeneous distillates are handled without difficulty.

This type of distilling head has the following advantages:

It gives a constant reflux ratio regardless of distillation rate Almost any reflux ratio can be obtained by proper design.

It requires no attention while in operation.

It has no moving parts or auxiliary equipment. It does not have excessive holdup and its cost of construction is low.

It can be operated under vacuum or pressure, and can handle heterogeneous as well as homogeneous condensates.

It can be readily built by a skilled glass blower without recourse to special tools or parts.

The number of reflux ratios obtainable with a single head is limited by the number of downspouts that can be built into the head.

ACKNOWLEDGMENT

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Laboratory Study of Continuous Vegetable Oil Extraction

Countercurrent Extractor, Rising-Film Evaporator, and Oil Stripper

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Apparatus useful for studies of continuous vegetable oil extraction in the laboratory are described in a continuous countercurrent extractor capable of supplying essential data, such as completeness of oil extraction, contact time, solvent-to-solid ratio, miscella composition, and solvent carry-over; a rising-film evaporator of the natural circulation type provided with a separator for continuous oil removal; and a new and efficient oil stripper providing turbulence and thin films by operating against gravity and against surface tension.

THE development of apparatus for the laboratory study of continuous countercurrent extraction of vegetable oils with ethanol stems from difficulties encountered in obtaining pertinent information by the usual batch-extraction methods both in the laboratory and in the pilot plant. These difficulties include the lack of information regarding the requisite ratio of solvent to solid, proper contact time between them in the extraction step, the deleterious effect on the oil and by-products caused by prolonged heating encountered in batch concentration, and the difficulties due to overheating and foaming encountered in stripping the last portions of solvent from the oil.

These difficulties are discouraging to anyone attempting to evaluate in the laboratory any solvent for use in oil extraction. However, the value of results from laboratory-size equipment capable of continuous operation is of course enhanced when essential details of plant operation are duplicated, since, as has been pointed out (*2*), the "results obtained from one (laboratory-scale process equipment) carefully operated are probably more accurate than those obtained in most commercial operating tests". In an endeavor to achieve this goal, equipment was devised and trial periods of actual laboratory use were given to it. The pieces of equipment described here give data which are comparable to commercial operation. However, some of them are new and further work is needed to establish the feasibility of their large-scale operation.

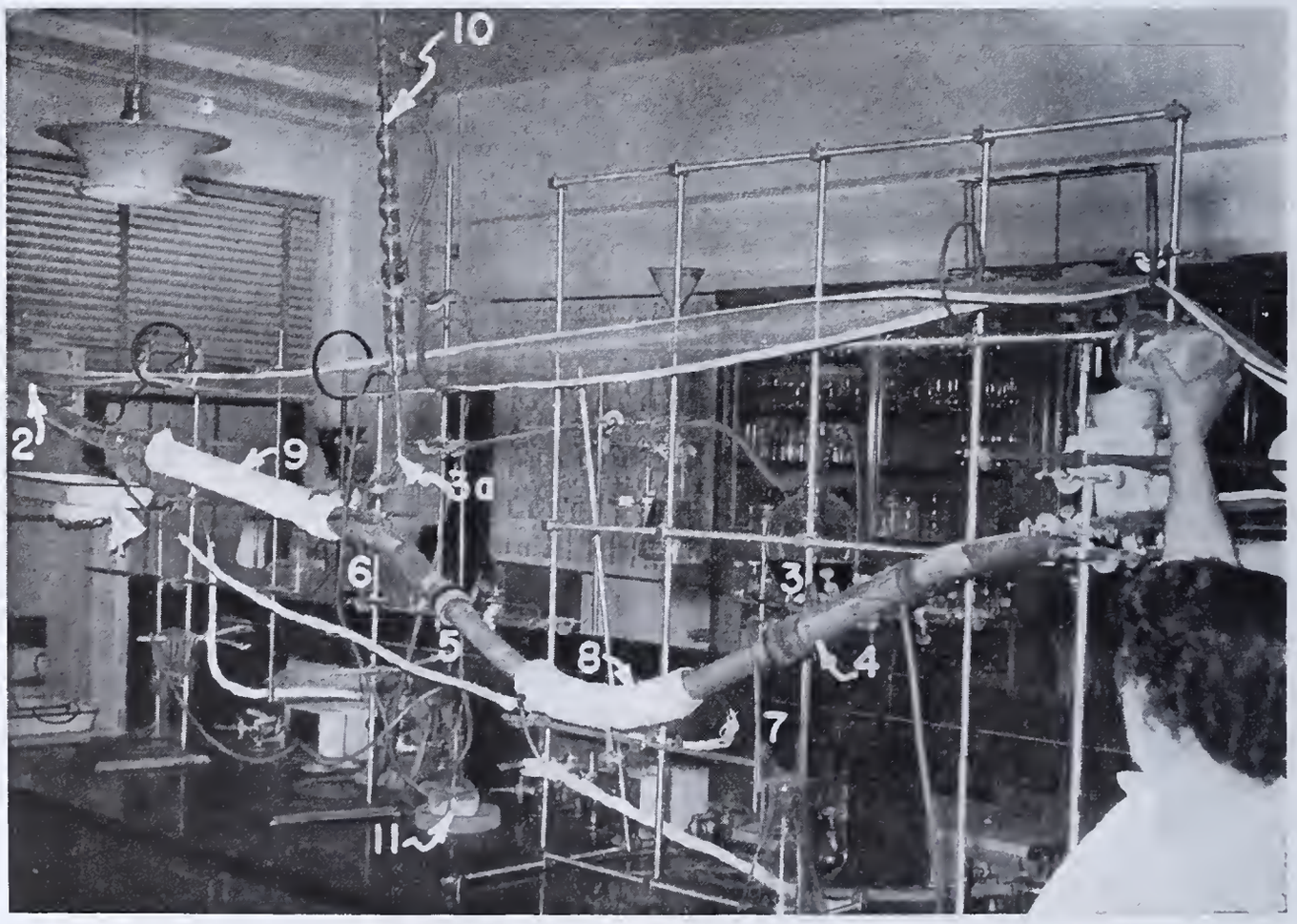

Figure 1. Continuous Countercurrent Vegetable Oil Extractor

The essential details of the equipment may be considered as a group of three divisions which may be designated as the extraction unit (including the dryer), the solvent- and by-product-recovery unit, and the oil-stripping unit.

In order to accumulate information for a materials balance, the extraction unit must contain means for uniform input of solvent and oil-bearing solid and control over products to permit analytical determination of the effect of the extraction. Furthermore, the solvent and solid feed must be variable over such ranges as to permit the extraction study to include significant solvent-to-solid ratios. Variability of the speed of the solids conveyor is also essential for determination of the proper time of contact between solvent and oil-bearing solid.

Important to the separation and recovery of the major fraction of the solvent is the effect of heat on the dissolved substances. This effect varies with the variety of oil extracted, since some oils undergo changes, such as permanent darkening, more readily than others. The heat effect varies also with the solvent used, since that determines the quantity and character of material other than oil which is dissolved. In any case, heating of prolonged duration, such as occurs in the pot-still type of batch concentration, is avoided in commercial practice by using the falling film, packed column, or rising-film type of evaporator where the liquid in the process of evaporation passes rapidly over the heated surfaces.

The effects of heat receive even greater emphasis when the solvent remaining after evaporative concentration is stripped from the oil. This residual solvent can be removed under ordinary pressures only at relatively higher temperatures than prevail in the first stage of concentration (*3*). By sweeping with steam or an inert gas under reduced pressures, the temperature can be greatly reduced, and this procedure is usually followed with the falling-film type of stripper, the bubble-cap tower, or the packed column used as the stripping apparatus. The new apparatus described for stripping solvents from oils may be evaluated in terms of the following discussion.

The process of stripping proceeds with greatest rapidity when thin films are presented to the heating surfaces and to the stripping atmosphere. Under such condition, heat transfer and diffusion of solvent-to-free liquid surfaces take place most rapidly. The production of thin films in any equipment in which the liquid flows freely over a surface is complicated by the surface tension forces which cause the liquid to attempt to occupy the volume having the smallest surface area, with the result that frequently the falling film becomes a meandering trickle of appreciable thickness. Similarly, capillarity, because of the proximity of surfaces in packed columns, partially circumvents the production of thin films. This latter effect leads also to channeling of the descending liquid and of the rising atmosphere.

EXTRACTION UNIT

A photograph of the continuous countercurrent extraction unit including the meal dryer is shown in Figure 1. Figure 2 is described as to construction and operation by considering the conveyor system, solvent system, and drying system, in that order.

The conveyor is made from ordinary 16-mesh galvanized iron window screen of the Pearle type by cutting a strip 12.1 cm. (4.75 inches) wide by 8.53 meters (28 feet) long and binding the edges with cloth to prevent raveling. It is made continuous by lapping the ends on a bias cut and stapling them with a paper stapler. In operation, it has been far more satisfactory and durable than originally anticipated, since the present conveyor has had an almost daily use of 6 or 7 hours for about 10 months and will evidently survive many months more.

In conveying the oil-containing solid through the body of the extractor, which consists of 45-mm. tubing bent in the shape shown, the screen closes around the solid completely, and it is essential that the quantity of material be sufficient to fill the tube, or the solvent will channel past too freely. The charging operation is shown at 1, where a weighed quantity of material is fed into a hopper which is maintained at a constant height above the moving screen, thus supplying a uniform feed to the extractor. A tabulation of the quantity of solid required to maintain a given level of material in the hopper supplies information as to the grams fed per hour. The rate of solid feed is regulated by the speed of the conveyor, and that, in turn, is regulated by the time of contact desired between the solid and solvent. The screen belt conveyor is pulled through the system by passing the belt between rubber rolls 2, which are driven by a variable-speed motor (not shown) through speed-reducing gears, so that any contact time from 0.5 hour up to more than 1.5 hours may be obtained.

Table I. Performance of Oil Extractor

Solvent	Temperature	Solvent-to-Solid Ratio[a] (Weight)	Contact Time, Min.	Oil Left in Residue, %
Hexane (Skelly B)	Room	1.2/1	55	0.39
Ethyl alcohol (absolute)	Boiling	2.12/1	65	0.62
Isopropyl alcohol (absolute)	Boiling	1.27/1	65	0.54

[a] Feed rate of flakes 267 grams per hour.

The solvent is metered to the extractor by a small gear pump driven by a variable-speed motor through speed-reducing gears. Since the gear pump would not meter nonlubricating liquids, it was used to meter lubricating oil into a closed container, and the displaced air was used to meter solvent to the extractor. This displacement system is shown at 3 with the point of entrance of the solvent into the extractor at 3*a*. The solvent maintains a liquid level in the extractor from 4 to 5. From 5 to 6, miscella (solution) drain back and fresh solvent wash takes place, while the miscella outlet is located at 7. The outlet take-off tube is joined to the top side of the extractor tube to permit settling of fines. Hot extractions may be carried out by passing steam through a neoprene coil, 8, surrounding the center section of the extractor.

The major portion of solvent remaining on the extracted solid is removed in dryer 9, which is constructed of 65-mm. glass tubing in order to accommodate copper heating coils in addition to the conveyor belt and its charge. At the end of the dryer nearest the extractor, the size of the tubing is reduced to 45 mm. in order to make a smooth and tight connection to the extractor. At a point just before the reduction in size of the tubing, a short piece of 20-mm. tubing ending in a male 24–40 ground-glass joint is

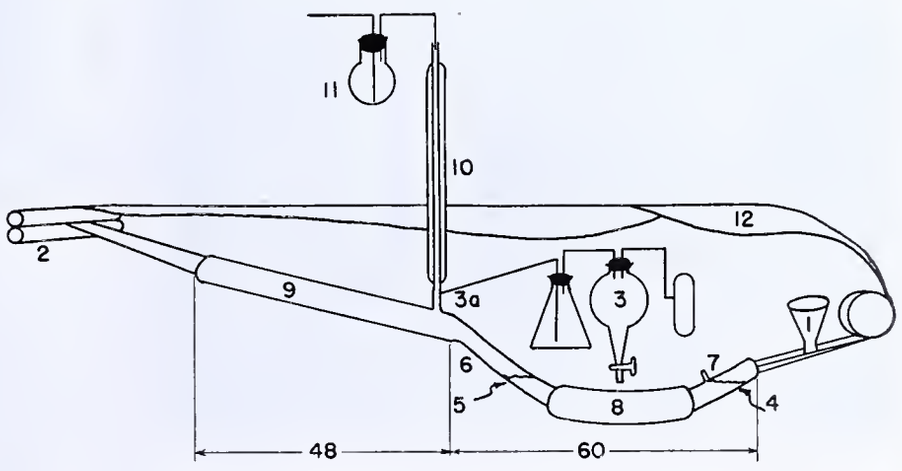

Figure 2. Continuous Countercurrent Vegetable Oil Extractor

1. Solid feed
2. Drive rolls
3. Solvent feed
4 to 5. Liquid level
5 to 6. Drainback and solvent wash
7. Miscella outlet
8. Heating coil
9. Dryer
10. Condenser
11. Trap to aspirator

joined to the dryer. This line is continued to a junction with the solvent inlet, 3*a*, and on to condenser 10, which in turn is connected through wash bottle 11 to a water aspirator. This aspirator is adjusted to cause a very gentle movement of air over the heated solvent-wet solid in the dryer and up through the condenser, where the entrained solvent vapors are condensed and flow down with the solvent feed into the fresh solvent wash portion of the extractor. The conveyor turns over during passage through the dryer and, on emerging, opens up to discharge the dried solid into a tray.

The efficiency of the extractor for extracting soybean oil is clearly indicated in Table I. Commercial-scale operation is predicated on leaving a residual oil content of 1% or less. Vapors of solvent in use are not detectable around extractor.

Information required for a materials balance can be obtained with this apparatus, as well as the solvent-to-solid ratios and contact times involved in reducing the oil content any desired amount.

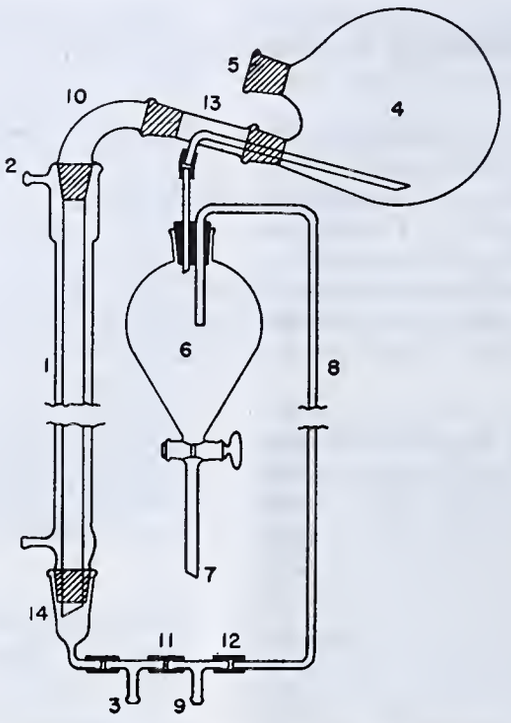

Figure 3. Continuous Natural Circulation Concentrator

1. Heating surface
2. Steam inlet
3. Solution inlet
4. Disengager
5. Disengager vapor outlet
6. Separator
7. Oil take-off
8. Return line
9. Solution take-off
10. 75° angle adapter
11, 12. Rubber connections
13. Disengager outlet
14. Reducing adapter

CONTINUOUS NATURAL CIRCULATION CONCENTRATOR

The rising-film evaporator, capable of continuous operation with natural circulation, is illustrated in Figure 3 and was assembled from standard glass equipment listed in glass-equipment catalogs. This availability, the versatility offered by a separator in the system, and the ease of conversion to an oil stripper are advantages over a setup (*1*) requiring construction of special units.

In the evaporating unit, steam is introduced at 2 into the outer jacket of a West-type condenser, 1, so that when the solution, which is fed into the bottom of the condenser through reducing adapter 14, reaches the heated surface, the sudden formation of solvent vapors causes it to be thrown violently up the condenser and through the 75° angle adapter, 10, and the gas-inlet adapter, 13, into the two-necked, 2-liter, round-bottomed flask serving adequately as a centrifugal disengager. This oil and unevaporated solvent are led into the 1-liter separatory funnel, 6, which is of importance in the concentration of partially miscible liquids such as alcohol and soybean oil. In this instance, at the temperature of the concentration, the oil separates in large drops which settle into a clear oily layer at the bottom of the separator, while the supernatant solution is led by line 8 back to the evaporator. The vapor from the disengager is led through 5 to a condensing system (not shown) which, in the authors' apparatus, consists of two condensers, and then into a solvent receiver which is connected to a vacuum line. The continuous concentrated solution take-off, 9, as well as the oil take-off, 7, must lead into vacuum chambers which operate under the same or lower pressures than the evaporator. To obtain the temperature to which the oil is heated, an adapter is available which has a thermometer inlet, and this may be substituted for the 75° angle adapter, 10. The removal of ethyl alcohol from an alcoholic soybean oil solution takes place in this apparatus at a rate of about 1800 ml. per hour under a pressure of 12 to 24 cm., depending on the concentration of the solution, and with the steam at atmospheric pressure.

Heat-transfer efficiency for a concentration may be calculated from the temperature of the steam, the temperature of the solution, the latent heat of vaporization of liquids, the area of the heating surface, and the rate of distillation of the solvent. Foaming is not a problem with this evaporator.

CONTINUOUS OIL STRIPPER

In order to avoid the previously described difficulty of obtaining films with the usual oil strippers, a stripper was devised which can be assembled from standard glass equipment or utilize the apparatus described in Figure 3.

In utilizing the latter apparatus, it is necessary only to put regulatory clamps over the rubber connections, 11 and 12. The clamp at 12 is entirely closed, and that at 11 is used to regulate the incoming flow of inert stripping atmosphere introduced at 9. The oil solution to be stripped is introduced at 3.

Stripping atmosphere and oil solution are introduced at the bottom of a tube which offers the only means of escape for the atmosphere, and, therefore, the gas rises through the tube as a series of bubbles, carrying with them a quantity of liquid. The stripping atmosphere is introduced too rapidly for the operation to be similar to the usual air lift, and, therefore, the quantity of liquid separating the bubbles is small, and their rise is very rapid, causing the liquid to spread on the surface of the heated tube until, finally, the thin bubbles break. This effect may be controlled, so that bubbles with film surfaces are continuously forming and breaking against the sides of the tube, in such a fashion that the liquid appears to be pushed against the sides in thin layers and, finally, is thrown over the top of the tube. As observed through the glass tubes, the operation takes place in a complex manner which bears no resemblance to the normal action of an air lift nor of a rising-film evaporator. The action supplies a force in active operation against the surface forces which come into control when a liquid flows freely over a surface, and thereby this stripper acts to overcome the contractile effect produced by those forces.

Since relatively large surfaces of solution are exposed, the stripping action has been found very successful. For example, the authors have been able to reduce the solvent content of an alcoholic soybean oil solution from 5 to 0.04% by operating the system under a pressure of 60 cm. with the heating tube and stripping atmosphere heated with steam at atmospheric pressure. A solution containing trichloroethylene, which is one of the most difficult solvents to remove from vegetable oils, was reduced from 7.0 to 0.04% in one passage. In this latter case, the oil showed no evidence of having been overheated.

The above-described equipment is now in use on a study of oil extraction of soybeans with ethanol and the results of this study will be published soon.

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Tropical Testing Chamber

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Figure 1. Interior View of Tropical Testing Chamber

THE tropical testing chamber of the Engineer Board was established at Fort Belvoir in the latter part of 1944 because the Corps of Engineers needed to test the resistance to tropical deterioration of a large number of items of equipment, as cork, fabrics, optical glass, leather, protective coatings, plastics, rubber (both natural and synthetic), wood and wood products (such as paper), and materials used in electrical and electronic equipment and in mapping equipment.

The tropical testing chamber may be considered analogous to a pilot plant in industry. In such an analogy, a pure culture test would be comparable to a laboratory batch in industry, and a tropical field test comparable to a plant batch. In the large tropical chamber end items of equipment can be tested, which is particularly important in the case of such matériel as optical instruments and electrical and electronic equipment.

The tropical testing chamber is 14 feet in width, 32 feet in length, and 12 feet in height. The wall construction consists of three layers, an outer layer of brick 9 inches thick, a middle layer of asphalt-impregnated mineral wool 4.5 inches thick, and an inner layer of Transite 0.375 inch thick. The outer layer of the ceiling is an asphalt slag roof; next is a layer of Celotex, 1.5 inches thick; next, a metal deck; under the metal deck is a layer of asphalt-impregnated mineral wool 6 inches thick; under the mineral wool is a 2-foot air space for circulation of air; and finally, a Transite ceiling 0.375 inch thick. The chamber has double doors which are 9 feet wide and 11 feet high and a single pedestrian door. The humidity and temperature within the room are controlled by heating and cooling coils through which the air is blown. A fan produces an air movement with a velocity of 4 to 5 miles an hour. Water vapor sprays are provided at the back of the coils.

Figure 1 is a photograph of the interior of the chamber taken through the double doors, showing the heating and cooling coils at the rear. On one side are wooden shelves and on the other is a bench with two additional shelves below it. At the end of the chamber opposite the large double doors are three soil burial beds 4 feet wide by 7 feet long by 10 inches deep. One of the beds is mounted on rollers over the other two beds. On the floor is abundant leaf mold, providing a source of food for the fungi. Wooden walkways make all parts readily accessible. Figure 1 shows an electrical generator set and two rolls of asphalt-coated burlap being exposed on the floor.

A definite temperature and humidity cycle is maintained in the tropical testing chamber. For 18 hours, the relative humidity is maintained at 90 ± 2% and the temperature is held at 85° ± 1° F. For 6 hours, the relative humidity is maintained at 95 ± 2% and the temperature is held at 75° ± 1° F. This cycle is shown graphically in Figure 2.

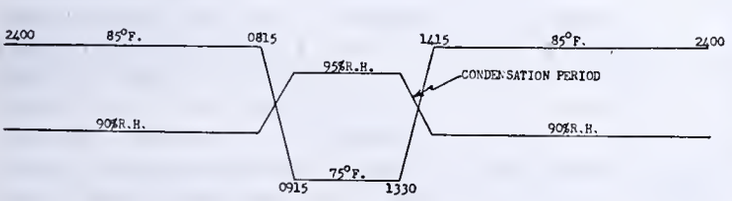

Figure 2. Temperature-Humidity Cycle of Testing Chamber

Figure 3. Samples Being Exposed in Testing Chamber

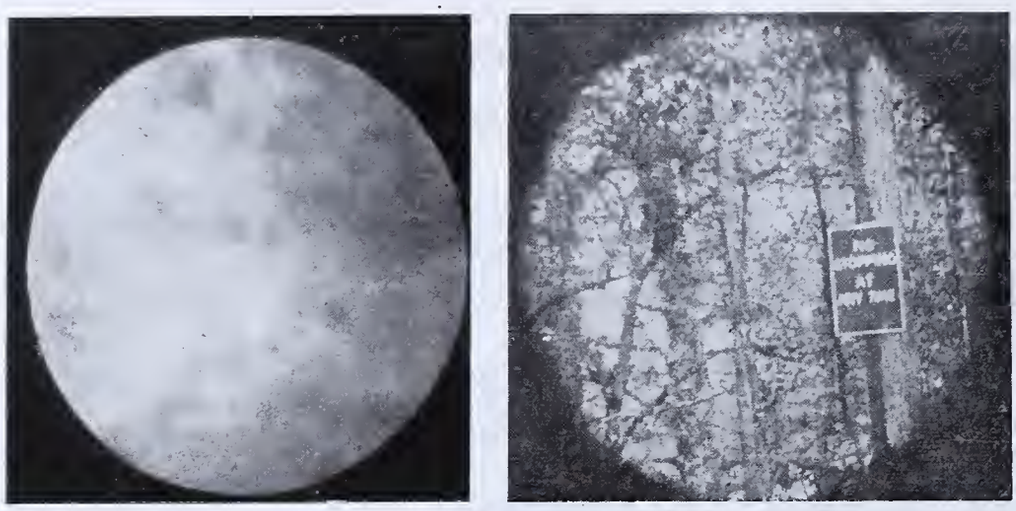

Figure 4. Photographs Taken through Telescopes after 2 Months' Exposure in Chamber

Left. **Untreated telescope.** ***Right.*** **Telescope treated with radium foil**

Table I. Fungi Introduced into Tropical Testing Chamber

Species	Source	Species	Source
Chaetomium globosum	Local	*Penicillium sp.*	India
Aspergillus niger	Hollandia	*Penicillium expansum*	Local
Trichodermo lignorum	South Pacific	*Penicillium sp.*	New Guinea
Aspergillus oryzae	South Pacific	*Aspergillus oryzae*	China
Mucor sp.	South Pacific	*Penicillium sp.*	South Pacific
Penicillium sp.	South Pacific	*Chaetomium sp.*	New Guinea
Aspergillus 19B	South Pacific	*Aspergillus niger*	South Pacific
Memnoniella echinata	New Guinea	*Aspergillus niger*	Local
Penicillium luteum	India	*Spicarios sp.*	South Pacific
Stemphylium sp.	New Orleans	*Chaetomium sp.*	South Pacific
Memnoniello sp.	South Pacific	*Mucor sp.*	South Pacific
Metarrhizium glutinosum	New Orleans	*Aspergillus versicolor*	South Pacific

A relative humidity of 90% at 85° F. is ideal for the growth of most fungi and is typical of tropical conditions.

A relative humidity of 95% at a temperature of 75° F. was chosen because it provides a temperature drop and a relative humidity rise comparable to conditions which occur in the tropics.

Cycling provides breathing for partially closed systems such as optical instruments.

Cycling causes condensation which provides a film of water ideal for fungus growth and excellent conditions for the corrosion of metals.

The chamber was inoculated by the addition of spore suspensions of 24 species of fungi obtained chiefly from the Pacific area, which were disseminated by means of spray, and by the native fungi occurring on the leaf mold placed on the floor of the chamber. The list of the 24 species of fungi is given in Table I. Other fungi were undoubtedly brought into the chamber by the air and by test specimens.

Wooden shelves are employed for the shelf exposure of materials because wood provides a source of nutrient for fungi, and also because exposure on wooden shelves duplicates storage conditions in the field. Such items as fabrics, small pieces of cork, etc., are hung from glass rods with glass hooks. Packaged materials and large objects are placed directly on the shelves. Figure 3 provides a close-up of test specimens being exposed in and on the wooden shelves. The center of the chamber is used for the exposure of large pieces of equipment. The soil burial beds in the room provide soil exposure tests which are likely to be more reproducible than usual because of the close control of the water content and temperature of the soil made possible by the controlled humidity and temperature in the chamber.

The advantages of tropical chamber exposures are:

Samples are exposed under conditions approximating those in nature.

Samples are constantly reinoculated with fungus spores.

Samples are inoculated by a wide variety of species of fungi, so that after one grows, others may follow, if a breakdown of the fungicide is caused by the first fungi.

The end item is exposed in its entirety.

Samples are exposed to deterioration caused by moisture from condensation.

Samples are exposed to deterioration aided by mites and other insects which are present in the chamber (mites are desirable in testing such items as optical instruments).

Samples are exposed to deterioration caused by bacteria.

It is desirable to correlate the results of tropical chamber tests with other types of testing. Comparisons with tropical field tests and with soil burial and pure culture tests are being made. As part of a study of commercially available fungicides applied to fabrics that is now being carried out at the Engineer Board, ten thousand fabric samples are being sent to the Panama Field Station of the National Defense Research Committee, and the results of these tests will be correlated with the results obtained from similar samples exposed in the chamber. Cork, leather, and optical instruments will also be exposed in the chamber at Panama and the results compared.

The fungi that are now present in the tropical testing chamber are being identified. Eight of the species originally introduced have already been recognized as still present and several others which were probably introduced with the leaf mold have been identified.

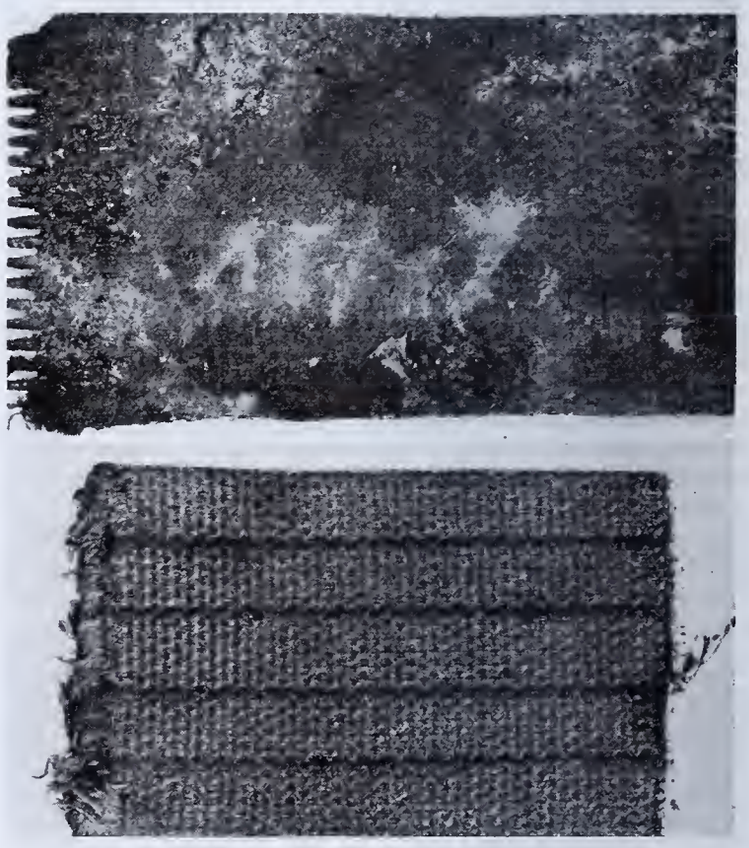

Figure 5. Cotton Sawmill Belting after 2 Months' Exposure in Chamber

Upper. **Untreated belting**
Lower. **Belting treated with copper naphthenate and pyridyl mercuric stearate**

The serviceability and usefulness of tropical testing chamber exposures are illustrated by Figures 4 and 5. Figure 4 contains photographs taken through an untreated telescope and one treated with radium foil (a treatment developed at the Engineer Board) after 2 months of exposure in the chamber. Figure 5 shows a comparison of a sample of untreated cotton sawmill belting and one treated with copper naphthenate and pyridyl mercuric stearate after two months of exposure in the chamber.

Semimicro-Kjeldahl Procedure for Control Laboratories

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NITROGEN analyses must often be run either on a very limited amount of sample or in the shortest possible time. While the usual macroprocedure is not satisfactory under these conditions, micromethods often cannot be employed because of limitations on equipment and personnel. In recent years sufficient improvement and simplification have been made in the semimicromethod to permit its use in almost any laboratory. Among the modifications which have been made are the distillation unit of Redemann (*4*), the adaption of the Winkler boric acid method to the semimicro scale by Wagner (*6*), the introduction of an improved indicator for use in presence of boric acid by Ma and Zuazaga (*1*), and the discovery of highly efficient digestion catalysts of the mercury-selenium type by Osborn and Krasnitz (*3*).

Table I. Determination of Nitrogen in Simple Compounds

Compound	% Nitrogen Found	Theory
Acetanilide	10.44 10.34	10.36
p-Bromoacetanilide	6.47 6.43	6.54
Benzanilide	7.14 7.07	7.10

This paper describes a procedure which incorporates these recent modifications and may be used with equipment available in almost any laboratory. The procedure has been checked by two different laboratories and has given excellent results for more than 3 years.

APPARATUS

The balance employed should be adjusted to give a precision of 0.1 mg. or better when weighings are conducted by the method of swings. All weights used should be checked to assure accuracy within the precision of the balance.

Samples are introduced into the digestion flask by means of a long-stemmed weighing tube (*2*) to prevent errors resulting from particles of sample adhering to the neck of the flask and escaping decomposition.

A digestion rack can be made by attaching six buret clamps to an iron bar which is clamped to a ring stand. The 100-ml. Kjeldahl flasks are heated with a Bunsen or Fisher burner.

The Redemann distillation unit can be made by a glassblower of ordinary skill or may be purchased from Scientific Glass Apparatus Co., Bloomfield, N. J., catalog No. M-1285.

REAGENTS

Catalyst Mixture. Grind together 150 grams of anhydrous potassium sulfate, 5 grams of metallic selenium, and 10 grams of mercuric oxide.

Mixed Indicator. Prepare 0.1% bromocresol green and 0.1% methyl red solutions in 95% alcohol separately. Mix 10 ml. of bromocresol green solution with 2 ml. of methyl red solution.

Boric Acid, 4%. Dissolve 20 grams of boric acid in 500 ml. of boiling distilled water.

Sodium Hydroxide, 48%. Dissolve 480 grams of sodium hydroxide in 520 ml. of distilled water and allow to stand until carbonate-free.

Sodium Thiosulfate, 44%. Dissolve 88 grams of sodium thiosulfate pentahydrate in 112 ml. of distilled water.

Standard Hydrochloric Acid, 0.015 *N*. Prepare a 0.015 *N* solution and standardize against pure sodium carbonate, using methyl red as an indicator.

PROCEDURE

A 15- to 50-mg. sample (30 to 50 mg. for balance with precision of 0.1 mg., 15 to 25 mg. with precision of 0.03 mg.) is weighed into a 100-ml. Kjeldahl flask, and 4 ml. of concentrated sulfuric acid and about 1.5 grams of catalyst mixture are added. Digestion is started with a low flame, gradually increasing the heat until the mixture boils briskly. For most samples the solution becomes clear after about 10 minutes heating. Digestion is continued for 25 minutes after clearing; for difficult compounds such as pyridine derivatives the afterboil should be extended to 1 hour. The flask and contents are cooled to room temperature, 20 ml. of water are added, and the solution is well mixed.

A blank flask is fitted into place in the distillation unit and the apparatus is well steamed. The blank flask is replaced by the flask containing the sample and a moderate current of steam is passed over until the apparatus is completely filled with steam. A 125-ml. Erlenmeyer flask containing 10 ml. of 4% boric acid solution and 3 drops of mixed indicator is placed under the delivery tube, with the tip of the delivery tube below the surface of the acid. During the course of several minutes 12 ml. of 48% sodium hydroxide solution are slowly added. Dropwise addition of base is necessary until most of the acid is neutralized to prevent too violent a reaction. About 4 ml. of sodium thiosulfate solution are added immediately after the base and the solution is steam-distilled as rapidly as possible. After 25 to 30 ml. of distillate have been collected, the receiver is lowered and about 5 ml. of additional distillate are collected.

Table II. Determination of Nitrogen in Ring Compounds

Compound	% Nitrogen Found	Theory
Pyridine hydrochloride	11.99 11.93	12.12
2-Aminopyridine	29.54 29.55	29.76
2-(Pyridyl-2) ethyl phenyl amine	14.06 13.90	14.13
2-Chloroquinoline	8.54 8.53	8.57
Triphenyltriazine	13.46 13.52	13.51
2-Benzoyl-5-phenyl-glyoxaline	11.27 11.23	11.29
2,5-Diphenyl-3-keto-3,4-dihydropyrazine	11.33 11.24	11.29
2-Methyl-4-phenyl-5-keto-dihydroglyoxaline	15.93 16.10	16.09

The boric acid solution changes from pink to a bluish green as soon as it comes in contact with ammonia. The solution is titrated with 0.015 *N* acid. Since the true end point is difficult to detect, the titration is continued until a faint pink color appears. The volume of acid required to produce a pink color of this same intensity is determined by adding standard acid to a solution of the same volume, containing the same quantity of boric acid and indicator. The blank (usually about 0.20 ml. of 0.015 *N* acid) is subtracted from the volume of acid required to titrate the sample.

Table III. Determination of Nitrogen in High Polymeric Materials

Polymer	% Nitrogen Found	Calculated[a]
Butadiene-acrylonitrile 70/30 copolymer	7.69 7.64	7.65
Butadiene-acrylonitrile 60/40 copolymer	9.15 9.06	9.14
Butadiene-2-vinylpyridine 75/25 copolymer	3.26 3.25	3.27
Butadiene-2-vinylpyridine 60/40 copolymer	5.16 5.19	5.00
Butadiene-2-vinylpyridine 50/50 copolymer	6.19 6.32	6.27
Chloroprene-2-vinylpyridine copolymer	1.34 1.32	1.30
Polymeric salt of sebacic acid and *p*-xylylenediamine	8.02 8.13	8.28
Polyhexamethylene adipamide (nylon)	12.03 11.99	12.11

[a] Calculated values were obtained from analysis of polymer by an independent method such as Dumas nitrogen or determination of carbon-hydrogen ratio.

RESULTS

Table I indicates that the procedure gives excellent results.

With the exception of compounds which contain a nitrogen to nitrogen or a nitrogen to oxygen linkage, practically all organic nitrogen compounds can be analyzed by the Kjeldahl method. So far the authors have encountered no exceptions to this rule. In Table II analytical results are listed for a number of nitrogen ring compounds including several pyridine derivatives. For pyridine compounds the digestion time had to be extended to about one hour. Shirley and Becker (*5*) observed that the use of a copper sulfate catalyst gave low results with pyridine-type compounds while correct results were obtained with a mercury or mercury–selenium oxychloride catalyst. This observation has been confirmed by the authors.

The literature contains little information as to the reliability of the Kjeldahl method for high polymeric materials. All high polymeric materials investigated by the authors to date have given the correct results by this method. However, the same limitations as to structure should apply for high polymers as for the usual compounds. Table III gives analytical results for a number of high polymeric substances.

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Sensitive and Selective Test for Gallotannin (Tannic Acid) and Other Tannins[1]

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A VIOLET flocculent precipitate is slowly produced when an ammoniacal solution of tannic acid is warmed with a solution of ferrous α,α'-dipyridyl sulfate, $Fe(\alpha,\alpha'\text{-dip})_3SO_4$. The precipitate, which filters well, forms quickly if the ammoniacal solution or suspension is treated with acetic acid and warmed. Precipitation is complete, as shown by the negative response of the filtrate to the most common tannic reagent—i.e., ferric chloride plus sodium acetate (*2*). A solution of ferrous α,α'-phenanthroline sulfate, $Fe(\alpha,\alpha'\text{-phen})_3SO_4$, shows a similar reaction toward tannic acid.

Attempts to isolate precipitates of constant composition have not been successful. Nonetheless, the chemical basis of this new reaction of tannin deserves consideration. Some of the factors involved are: the phenolic nature of the tannin, the colloidal character of aqueous tannin solutions, the autoxidation of these solutions at pH greater than 7, and the ability of $Fe(\alpha,\alpha'\text{-dip})_3^{++}$ ions to combine with voluminous and complex anions to form red, slightly soluble salts (*1*).

Of the possible types of reaction, the writers believe the most probable to be the formation of an adsorption complex by combination of the oxidation products of the tannin with Fe $(\alpha,\alpha'\text{-dip})_3(OH)_2$, or $Fe(\alpha,\alpha'\text{-phen})_3(OH)_2$. Adsorption compounds of tannin with hydrous metal oxides have been reported and used for analytical purposes (*3*, *4*). The assumption that it is not tannin itself, but rather an oxidation product (of unknown composition) which takes part in this reaction, is supported by the observation that no precipitate is formed if air is excluded, or if much alkali sulfite is present. This is also in agreement with the fact that the deposition of the violet precipitate occurs gradually, and the precipitate always forms first at the upper surface of the liquid. This effect is seen distinctly if the solution of tannic acid is not extremely dilute.

The probability that autoxidation of tannic acid is responsible for the reaction with $Fe(\alpha,\alpha'\text{-dip})_3^{++}$ ions made it likely that other autoxidizable phenolic compounds would exhibit an analogous behavior. Trials showed that ammoniacal solutions of pyrogallol give a very strong reaction. Gallic acid, in cold saturated (1%) solution, gives a visible response, though much less decided than tannic acid and pyrogallol at this dilution. Hydroquinone reacts weakly. Phloroglucinol, resorcinol, and pyrocatechol give no response. These findings led to the expectation that vegetable tannins would behave toward $Fe(\alpha,\alpha'\text{-dip})_3SO_4$ as tannic acid does, since they are all phenolic in nature, and their alkaline solutions are said (*2*) to absorb oxygen. This prediction has been realized with all the natural tanning agents that have been tested thus far, though the number of varieties available has not been large. Consequently, this reaction applies to gallotannin and to other tannins. As will be seen, the nature of the response to the tannin reaction with $Fe(\alpha,\alpha'\text{-dip})_3SO_4$ is not determined by whether the test material is a pyrogallol- or catechutannin. Synthetic tans, which mostly are condensation products of formaldehyde and sulfonated phenols, give no response.

[1] Translated from the German manuscript by Ralph E. Oesper, University of Cincinnati, Cincinnati, Ohio.

DETECTION OF TANNIC ACID (GALLOTANNIN)

Reagent. Dissolve 0.25 gram of α,α'-dipyridyl and 0.146 gram of ferrous sulfate heptahydrate in 50 ml. of water. The solution keeps well in closed containers. Before the test, it is well to render a portion of the reagent ammoniacal and boil. Remove the resulting slight precipitate of hydrous ferric oxide by filtering or centrifuging. This purification is essential when testing for small quantities of gallotannin or other tannins.

Procedure. *Test Tube Reaction.* Treat 1 ml. of the test solution with an equal volume of ammoniacal reagent solution and bring the mixture to boiling. Add acetic acid until the odor of ammonia vanishes and again heat the solution to boiling. A flocculent violet precipitate forms. If only minute quantities of the tannin are present, the precipitate has a brownish tinge. Turbidities produced by minute amounts of precipitate can be easily discerned in the red solution, if the test tube is held toward an intense source of light and a sheet of frosted glass interposed. Identification limit, 8 micrograms of tannic acid; limiting concentration, 1 to 125,000.

Drop Reaction. Place one drop of the test solution in a small (0.05-ml.) centrifuge tube, add 2 drops of reagent solution and suspend the tube in boiling water for several minutes. After acidifying with acetic acid, again warm the solution and then centrifuge. Any precipitate collects in the constricted end of the tube. Very tiny precipitates are readily seen if, after centrifuging, the tube is held against white paper. A blank test is recommended when small amounts of tannic acid are suspected. Identification limit: 1 microgram of tannic acid; limiting concentration: 1 to 50,000.

The test for tannic acid can alternatively be carried out as follows:

Make the test solution ammoniacal, warm, acidify with acetic acid, and then add the reagent solution. This procedure is not so sensitive as those already outlined.

The nonspecific phenol reaction of tannic acid with dilute sodium acetate–ferric chloride solution is not so sensitive as the test described here. As a test-tube reaction the identification limit is 25 micrograms; the limiting concentration is 1 to 40,000. The corresponding figures for the drop reaction on a spot plate are 1 microgram and 1 to 50,000. Hence the new test is three times as sensitive as the ferric-phenol reaction when carried out in a test tube, and just as sensitive as the latter when spot test techniques are used.

DETECTION OF NATURAL TANNINS AND DIFFERENTIATION FROM SYNTHETIC TANS

The test solution (0.5%) is prepared from the solid specimen. One milliliter of the filtered liquid is carried through one of the procedures as outlined. When testing extracts of tanning agents, test portions are prepared by diluting the specimen 1 to 10 and 1 to 100, and 1-ml. portions are used. The behavior of all solutions toward ammonia and acetic acid should be determined before adding the reagent. If a precipitate appears on adding acetic acid to the warm ammoniacal solution, it should be removed, and the test tube procedure carried out on the filtrate after again making the solution ammoniacal. The test with $Fe(\alpha,\alpha'\text{-dip})_3\text{-}SO_4$ was tried on a variety of tanning materials. The following natural tannins gave a positive reaction: gallotannin; tara powder; gambier; myrobalan; quebracho; and extracts of wattle, mangle, sumac, and fustic. In marked contrast the following synthetic tanning agents gave a negative reaction: orotan N, syntan A, syntan S, mertanol 7 L, maxyntan, and tanasol. These findings indicate that this test is suitable for distinguishing commercial natural from synthetic tanning agents.

Similar differentiating tests were also made on finished, mostly colored, leathers. About 0.5 gram of the leather was cut into tiny bits and boiled in 2 ml. of ammonia water for 2 minutes. The clear filtrate was carried through the test procedure. Preliminary tests with ammonia and acetic acid were made in all cases. This step is indispensable when testing leathers colored with coal-tar dyes, because the addition of the reagent to the ammoniacal solutions of many acid dyes results in a precipitate that does not dissolve in acetic acid. If the preliminary test produces a precipitate, it is filtered off, and the tannin test is made on the clear filtrate after it is again made ammoniacal.

In 19 out of 20 cases, the test revealed the nature (vegetable or synthetic) of the tanning agent. The single exception was a leather that had been tanned with a sulfited quebracho extract; it gave a negative response. Further studies will be necessary to determine whether the test fails with leathers tanned with strongly sulfited tannin extracts. A trial with a technical sulfited quebracho extract showed that it still gives a distinct tannin reaction at a dilution of 1 to 1000.

DETECTION OF TANNINS IN BEVERAGES

Three milliliters of the samples were taken for the tests. Positive reactions were given by 4 varieties of red wine (tart, sweet); 3 varieties of white wine (tart, sweet); and water extracts of tea, maté, and guarana. Negative responses were obtained with Cinzano (Italian origin); beer (light, dark); and water extracts of coffee (raw, roasted).

ACKNOWLEDGMENT

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Gas Bubble Releaser for Use in Dumas Nitrogen Determination Azotometers

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A RATHER common and annoying occurrence in running a Dumas nitrogen determination is the sticking of gas bubbles to the surface of the mercury in the azotometer. This has been attributed to too narrow an opening of the gas inlet of the nitrometer, too short a distance between the gas inlet and the level of the mercury, excessive greasing of the gas inlet stopcock (*3*), and the use of perfectly pure clean mercury when the azotometer is first filled (*4*).

Flaschenträger (*1*) and Weygand (*5*) say that this difficulty can be overcome by the addition of powdered copper oxide to the surface of the mercury. Nichols (*2*) suggests the use of mercurous oxide for the same purpose. None of these measures, which are preventive in character, has been found completely reliable.

A simple direct method for releasing gas bubbles already sticking to the mercury has been used effectively in this laboratory for over a year.

A piece of steel or iron wire about 1 cm. long, slightly curved, is placed in the azotometer so that it rests on the surface of the mercury. Whenever gas bubbles are to be released, the piece of wire is swept over the mercury by means of a small permanent-type magnet held outside the azotometer near the level of the mercury and opposite the wire. The wire is attracted to the magnet and, in passing over the surface of the mercury, releases the bubbles. The wire can thus be moved back and forth simply by placing the magnet in the proper position opposite it. To facilitate this operation two magnets can be used. These are held on opposite sides of the azotometer and brought near the azotometer alternately.

In addition, this device can be used to break up small bubbles at the potassium hydroxide solution-gas interface. This is done by attracting the wire to the magnet and moving it up slowly to the interface. By moving the wire up and down through the interface, the gas bubbles are broken. Large gas bubbles stuck or moving up very slowly in the graduated portion of the azotometer can be released also by this means.

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Determination of Reducing Sugars

Mathematical Expression of Reducing Action in the Lane and Eynon and Volumetric Ferricyanide Methods

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IN THE determination of reducing sugars by direct titration against Fehling solution according to Soxhlet, Violette, or Pavy, it has been generally assumed that the concentration, x, of sugar solution, multiplied by the volume, y, required for complete reduction of a fixed quantity of copper solution, is constant ($xy = C$). But Soxhlet (*6*) showed as early as 1878 that this simple relationship holds only if the concentration of the unknown sugar solution is approximately the same as that used for standardization of the Fehling solution. Later, Lane and Eynon (*4*) found that the "factor" ($C/100$) may vary as much as 5% for a range of 15 to 50 ml. of sugar solution used.

When it became apparent about two years ago that copper salts might be difficult to obtain, it was decided to devise a substitute for the Lane and Eynon method, and alkaline ferricyanide solution was tried, as first proposed by Ionescu and Vargolici (*1*), with methylene blue as indicator of complete reduction. When the results were plotted by Louis Sattler, of this laboratory, it was discovered that there is a straight-line relationship not between x and y, but between their logarithms, and that the results can be expressed by the equation

$$\log y = \log b - m \log x \qquad (1)$$

or

$$y = bx^{-m} \qquad (2)$$

where b and m are constants. The original equation, $xy = C$, or $y = C/x$, is a special case of the general equation $y = C/x^m$, with $m = 1$.

EQUATIONS FOR LANE AND EYNON METHOD

To test the validity of this law of reducing action for the Lane and Eynon method, the values of m and $\log b$ were calculated by the method of averages from 18 pairs of values for milligrams of sugar in 100 ml. of solution and the corresponding titers given in the Lane and Eynon tables (*3*). A detailed comparison between the figures in the table for invert sugar alone, with 10 ml. of Fehling solution, and those calculated from the equation is shown in Table I. In this particular case m was found to be 1.0341, and $\log b = 3.7886$.

Table I. Comparison between Lane and Eynon Titers and Those Calculated from Equation 1 for Invert Sugar Alone

(10 ml. of Fehling solution)

Sugar in 100 Ml.	Sugar Solution, L. & E.	Sugar Solution, Equation 1	Difference
Mg.	*Ml.*	*Ml.*	*Ml.*
336	15	15.00	0.00
298	17	16.98	−0.02
267	19	19.03	+0.03
242.9	21	20.98	−0.02
222.2	23	23.00	0.00
204.8	25	25.02	+0.02
190.4	27	26.98	−0.02
177.6	29	29.00	0.00
166.3	31	31.04	+0.04
156.6	33	33.03	+0.03
147.9	35	35.04	+0.04
140.2	37	37.03	+0.03
133.3	39	39.01	+0.01
127.1	41	40.98	−0.02
121.4	43	42.98	−0.02
116.1	45	45.01	+0.01
111.4	47	46.97	−0.03
107.1	49	48.92	−0.08
Average difference			±0.023
Maximum difference			−0.08

Table II. Constants in Equation 1 for Reducing Effect of Various Sugars, as Determined by Lane and Eynon

	m	$\log b$	$\frac{m}{\log b}$	Deviation Average	Deviation Maximum
				Ml.	*Ml.*
10 ml. of Fehling solution					
Invert sugar	1.0341	3.7886	0.2730	0.023	0.08
Plus 1 gram of sucrose	1.0166	3.7405	0.2718	0.013	0.04
Plus 5 grams of sucrose	1.0030	3.6848	0.2722	0.012	0.04
Plus 10 grams of sucrose	0.9830	3.6240	0.2713	0.067	0.21
Plus 25 grams of sucrose	0.9552	3.5308	0.2698	0.109	0.28
Dextrose	1.0354	3.7789	0.2740	0.017	0.04
Levulose	1.0315	3.7966	0.2717	0.023	0.06
Maltose hydrate	0.9759	3.8444	0.2512	0.008	0.03
Lactose hydrate	1.0010	3.8348	0.2610	0.057	0.14
25 ml. of Fehling solution					
Invert sugar	1.0124	4.1270	0.2453	0.018	0.05
Plus 1 gram of sucrose	1.0086	4.1130	0.2452	0.009	0.04
Dextrose	1.0121	4.1138	0.2458	0.022	0.05
Levulose	1.0108	4.1362	0.2444	0.016	0.04
Maltose hydrate	0.9597	4.1932	0.2289	0.024	0.05
Lactose hydrate	0.9750	4.1593	0.2344	0.016	0.04

In Table II the values of m and $\log b$, calculated as explained, are shown for all the sugars and sugar mixtures studied by Lane and Eynon, together with the maximum and average deviations of the calculated titer from that given in their tables.

In two of the equations the value of m is so close to unity that the formula $y = C/x$ could be used without serious error. The ratio of m to $\log b$ for invert sugar is about midway between the ratios for dextrose and levulose. With increasing quantities of sucrose added to invert sugar both m and $\log b$ decrease, m more rapidly than $\log b$, as shown by the ratio between the two.

The agreement between the titers given in the Lane and Eynon tables and those calculated is remarkably close, the average deviations being in most instances around 0.02 ml. or less and the maximum deviations well within 0.1 ml. Larger discrepancies are found in the case of invert sugar in the presence of 10 or more grams of sucrose, titrated against 10 ml. of Fehling solution. Lane and Eynon have pointed out that the total time of boiling has a pronounced effect on the reducing power of invert sugar mixed with large amounts of sucrose. The maximum discrepancies occur usually when the titer is very high, close to 50 ml.

An interesting case is presented by lactose, titrated against 10 ml. of Fehling solution. Here the factor, $C/100$, found experimentally by Lane and Eynon decreases between 15- and 30-

Table III. Invert Sugar Table for 10 Ml. of Alkaline Ferricyanide Solution, Diluted with 20 Ml. of Water

Invert Sugar	Titer: Invert Sugar Alone		Invert Sugar + 1 Gram of Sucrose		Invert Sugar + 3 Grams of Sucrose		Invert Sugar + 5 Grams of Sucrose		Invert Sugar + 10 Grams of Sucrose	
	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.
Mg.										
400	14.30	14.28	14.12	14.11	14.11	14.00	13.89	13.84	13.62	13.64
360	15.93	15.95	15.79	15.76	15.64	15.64	15.52	15.48	15.16	15.15
320	18.00	18.05	17.78	17.83	17.64	17.70	17.56	17.54	17.14	17.17
280	20.83	20.77	20.49	20.51	20.28	20.36	20.12	20.20	19.71	19.79
260	22.45	22.46	22.18	22.16	22.01	22.01	21.78	21.85	21.39	21.41
240	24.50	24.43	24.08	24.10	23.92	23.94	23.85	23.79	23.49	23.32
220	26.70	26.77	26.46	26.40	26.31	26.23	26.01	26.09	25.50	25.58
200	29.60	29.60	29.11	29.18	28.99	28.99	28.91	28.86	28.39	28.31
180	33.08	33.07	32.51	32.60	32.36	32.38	32.25	32.28	31.51	31.67
160	37.41	37.43	36.99	36.87	36.58	36.64	36.56	36.57	35.81	35.89

ml. titer, and then increases again up to 50-ml. titer. This would mean that in the lower range m is greater than unity, but in the higher range smaller than unity, and would explain the unusual discrepancies observed when the entire range is considered to have only one value for m. But it is also possible that the exception is only apparent and caused by experimental difficulties in maintaining a uniform rate of boiling and addition of sugar solution, which affects the results obtained with disaccharides more than it does with monosaccharides.

Jackson and Mathews (*2*) have called attention to the fact that the Lane and Eynon factors found by different operators or with different batches of Fehling solution may vary somewhat from those given by Lane and Eynon, and recommend that each analyst standardize his own analyses with solutions of the pure sugar. This can now be readily done by plotting two or more points on double log paper and drawing a straight line through them.

VOLUMETRIC FERRICYANIDE METHOD

In this method the concentrations of the potassium ferricyanide and potassium hydroxide were increased over those specified by Ionescu and Vargolici, in order that titers between 15 and 50 ml. of sugar solution might correspond to approximately 400 to 100 mg. of invert sugar in 100 ml. of solution, similar to the range of the Lane and Eynon method. A solution containing in 1 liter 56.000 grams each of potassium ferricyanide and potassium hydroxide answered these requirements. Of this solution, 10 ml. were transferred to a 250-ml. Erlenmeyer flask, and diluted with 20 ml. of water. The determinations were then carried out exactly as in the Lane and Eynon method, 5 drops of methylene blue indicator being added toward the end of the titration. In each case the incremental method of titration was used in the first experiment, and in the subsequent experiments almost all of the sugar solution was added at one time, and the titration completed by dropwise addition of the sugar solution. The titers were determined in this manner for solutions containing invert sugar alone, and in the presence of 1, 3, 5, and 10 grams of added sucrose in 100 ml. of solution.

It was found that the precision of the ferricyanide method is not as high as in the Lane and Eynon method. When the titer lay between 35 and 50 ml., duplicate tests often varied by 0.2 to 0.3 ml. It is therefore advisable with this reagent to keep the titer within 15 and 35 ml., as was done by Main (*5*) in his pot method with Soxhlet solution. The titers found in this range for invert solutions, containing 400 to 160 mg. of invert sugar in 100 ml., are shown in Table III.

The values of constants m and log b in Equation 1, calculated from the experimental titers in Table III, are given in Table IV, together with the ratios of m to log b and the average and maximum deviations of the calculated from the found values. The titers calculated from the equations are shown in Table III, next to the found values.

The average deviations of the found from the calculated values are slightly larger than the corresponding figures for the Lane and Eynon method, even though the titer range is only from 15 to 35 ml. The same is true of the maximum deviations except for the mixture of invert sugar with 10 grams of sucrose. It must be considered, however, that Lane and Eynon did not publish their original experimental values, and that those given in their tables are probably taken from smoothed curves. Nevertheless, the lesser precision of the ferricyanide method is indicated by the fact that neither the m nor the log b values in Table IV show a regular trend, contrary to those in the Lane and Eynon method. The ratio of m to log b, however, increases regularly with an increase in added sucrose, whereas in the Lane and Eynon method the trend is downward. On the whole, the Lane and Eynon method appears to be preferable.

Ionescu and Vargolici claim that a solution may contain as much as 30% sucrose in addition to 0.5% glucose without affecting the titer. The figures in Table III show that this is not generally true, but that sucrose does affect the reducing power of invert sugar, as in the Lane and Eynon method.

Table IV. Constants in Equation 1 for Reducing Effect of Invert Sugar upon Alkaline Ferricyanide Reagent in Absence and Presence of Sucrose

	m	log b	$\frac{m}{\log b}$	Deviation: Average	Deviation: Maximum
				Ml.	*Ml.*
Invert sugar alone	1.0520	3.8919	0.2703	0.043	0.07
Invert sugar plus 1 gram of sucrose	1.0481	3.8768	0.2704	0.049	0.12
Invert sugar plus 3 grams of sucrose	1.0497	3.8777	0.2707	0.043	0.11
Invert sugar plus 5 grams of sucrose	1.0603	3.9001	0.2718	0.049	0.08
Invert sugar plus 10 grams of sucrose	1.0639	3.9001	0.2728	0.073	0.16

SUMMARY AND CONCLUSIONS

When the titers, y, found in the determination of reducing sugar by the Lane and Eynon method, or by a similar direct volumetric determination with alkaline ferricyanide solution, are plotted against the concentrations, x, of the reducing sugar, the equation of the resulting curve is $y = bx^{-m}$, or $\log y = \log b - m \log x$, a straight-line equation in which b and m are constants. These constants have been calculated for all sugars and sugar mixtures used by Lane and Eynon, with both 10 and 25 ml. of Fehling solution, as well as for invert sugar and mixtures of invert sugar with sucrose, determined by means of an alkaline ferricyanide solution. The agreement between the titers calculated from the equations and those given in the Lane and Eynon tables is very close. The precision of the alkalien ferricyanide method is somewhat lower; best results are obtained if the titer range is kept within 15 to 35 ml. Sucrose affects the reducing power of invert sugar not only in the Lane and Eynon method, but also in the alkaline ferricyanide method.

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A System of Laboratory Evaluation

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THE increasing use of statistical methods in analytical laboratories is a recognition of the value of having a control on the analytical process, which in turn is a control on a process or material. In the presentation of methods it is now commonplace to include a statement of standard deviations or probable error. Such statistical data are of considerable value, particularly in the selection of methods for particular purposes. However, the practical value of an analytical method in industrial laboratories does not depend so much upon the inherent accuracy and precision of the method as measured in a methods investigative laboratory as it does upon the actual performance of the method in the control or plant laboratory where it is used by available personnel, under conditions which may include special requirements as regards speed, quantity, and other factors.

Many data, including standard deviations, biases, probable errors, or limits of error and precision, are based upon analytical data produced under conditions which do not include the pressure, tensions, and limitations to be noted in most modern industrial plant laboratories. It seems obvious that the best way to measure the practical effectiveness of a method is to evaluate the analytical data when the operators are not conscious that their data on a particular or specific sample will be checked.

Many schemes have been tried out to make such evaluations. The problem is specially important when several laboratories are concerned and it is desirable to have their work maintained on similar levels of efficiency. The circulation of standard samples among laboratories is of limited utility, since the samples are invariably recognized and are given special attention, thus giving little information as to the normal efficiency of the laboratory.

Many other metering or checking schemes have been used and, within limits, all yield useful data. One plan that has worked out successfully in Aluminum Company of America metal plants over a period of years is described in the following paragraphs.

Arrangements are made at each plant maintaining a laboratory to choose an individual not connected with the laboratory to select check samples. This individual goes to the laboratory and selects at random a sample which has been analyzed and reported. The sample which is selected, together with the reported analysis, is sent to the Analytical Division at Aluminum Research Laboratories.

At Aluminum Research Laboratories the sample is assigned for analysis to a chemist who is not informed of the plant laboratory results. When the analysis is completed, the results are compared with the plant results. If there is failure to agree within satisfactory limits, the chemist is told to repeat his analysis in multiple to determine data on an umpire basis. The agreement of results is judged by the permissible deviations given in Table I. The results obtained at Aluminum Research Laboratories are the basis from which permissible deviations are measured.

In considering this table it should be borne in mind that the permissible tolerances which have been set up obviously pertain to only one industry and should not be construed as necessarily or desirably applicable in the case of materials other than aluminum and aluminum alloys.

Table I. Allowable Deviations in Check Analyses Metals

Range of Content %	Allowable Deviation %	Range of Content %	Allowable Deviation %
0.00–0.03	0.01	7.01– 7.50	0.31
0.04–0.10	0.02	7.51– 8.00	0.32
0.11–0.20	0.03	8.01– 8.60	0.33
0.21–0.30	0.04	8.61– 9.20	0.34
0.31–0.40	0.05	9.21– 9.80	0.35
0.41–0.55	0.06	9.81– 10.40	0.36
0.56–0.70	0.07	10.41– 11.00	0.37
0.71–0.85	0.08	11.01– 11.60	0.38
0.86–1.00	0.09	11.61– 12.20	0.39
1.01–1.15	0.10	12.21– 12.80	0.40
1.16–1.30	0.11	12.81– 13.40	0.41
1.31–1.45	0.12	13.41– 14.00	0.42
1.46–1.60	0.13	14.01– 14.75	0.43
1.61–1.80	0.14	14.76– 15.50	0.44
1.81–2.00	0.15	15.51– 16.25	0.45
2.01–2.20	0.16	16.26– 17.00	0.46
2.21–2.40	0.17	17.01– 17.75	0.47
2.41–2.60	0.18	17.76– 18.50	0.48
2.61–2.80	0.19	18.51– 19.25	0.49
2.81–3.00	0.20	19.26– 20.00	0.50
3.01–3.30	0.21		
3.31–3.60	0.22		
3.61–4.00	0.23	And thereafter increasing at	
4.01–4.40	0.24	the rate of 0.01% for each	
4.41–4.80	0.25	additional 1.0% of constit-	
4.81–5.20	0.26	uent, reaching 1.00% at	
5.21–5.60	0.27	70%, and thereafter re-	
5.61–6.00	0.28	maining constant	
6.01–6.50	0.29		
6.51–7.00	0.30	70.00–100.00	1.00

The laboratories are scored in accordance with the percentage of agreements. The allowable deviations were originally set up on more or less an arbitrary basis, but have evolved to their present levels by adjustments based upon the actual records made by laboratories. In general, all permitted deviations are smaller today than when the table was set up more than 20 years ago. The deviations shown are those permitted between a laboratory working under the pressure of plant production and a central laboratory working in the absence of production pressure. The table, however, is used in other ways. If in a given laboratory the work of one analyst under plant conditions is checked by another analyst in the same laboratory, the allowable deviations given in the table are to be divided by 1.5. For duplicate results by the same analyst, the allowable deviations in the table are divided by 2.5.

In Table II a typical tabulation of check results for a 6-month period is shown. Because of the nature of the determinations made, the laboratories are listed in two groups.

Table II. Laboratory Check Analyses

(6-month period)

Laboratory	Determinations	Outside Limits	Percentage Agreement
	Class R Plants		
A	50	0	100.0
B	50	0	100.0
C	47	0	100.0
D	46	0	100.0
E	28	0	100.0
F	51	1	98.0
G	33	1	97.0
	Class FC Plants		
H	272	0	100.0
I	211	0	100.0
J	198	0	100.0
K	212	1	99.5
L	183	1	99.5
M	251	2	99.2
N	115	1	99.1
O	180	2	98.9
P	168	2	98.8
Q	166	2	98.8
R	155	2	98.7
S	148	2	98.6
T	133	2	98.5
U	199	4	98.0
V	255	10	96.1
W	198	11	94.4
X	105	8	92.4
	3454	52	98.5

The determinations checked cover the ordinary impurities and alloying elements met in current metal production. Laboratories V and W are special cases with somewhat more difficult work than that carried out in other laboratories.

The checking system described affords a ready means of comparing the effectiveness of laboratories doing the same type of work. Investigation of failures to check often reveals weaknesses of methods, faulty application of methods, inadequate personnel, or other curable troubles. The main value of the system is that it reveals on a comparative basis the quality of work done in the laboratories.

Anomalous Behavior of Methyl 12-Hydroxy-9,10-Octadecenoates in Rapid Iodine Number Determinations

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The methyl esters of ricinoleic, ricinelaidic, and O-propionylricinoleic acids quantitatively add iodine chloride from Wijs reagent in less than 1 minute. However, if mercuric acetate is present, methyl ricinoleate and ricinelaidate react with additional halogen, thus giving high iodine values, whereas the methyl O-propionylricinoleate behaves normally. This anomalous effect is due to the presence of the free hydroxyl group.

THE use of mercuric acetate catalyst to speed the rate of addition of Wijs reagent to nonconjugated, unsaturated double bonds constituted a decided improvement in the iodine number technique by allowing the attainment of quantitative addition in less than 3 minutes (*2*), in contrast to the standard method for the determination of iodine value (*1*) which requires 30 to 60 minutes. Norris and Buswell (*4*) found that Hanus reagent with mercuric acetate was even more satisfactory than the Wijs reagent for the determination of nonconjugated unsaturation.

Table I. Rate of Reaction with Wijs and Bromine Reagents
(Without Mercuric Acetate)

Time, Min.	Halogen Equivalent[a]	Iodine Value
A. 0.02568 *M* Methyl Ricinoleate, 0.06861 *M* Wijs		
1.0	1.998	81.16
20.0	2.016	81.89
30.0	2.021	82.07
B. 0.02411 *M* Methyl Ricinelaidate, 0.06861 *M* Wijs		
1.0	2.006	81.50
3.0	2.005	81.45
11.0	2.009	81.60
30.0	2.006	81.50
30.0	2.004	81.40
60.0	2.009	81.60
C. 0.02568 *M* Methyl Ricinoleate, 0.07449 *M* Bromine		
1.0	2.030	82.44
1.0	2.035	82.67
10.0	2.021	82.07
20.0	2.024	82.21

[a] Halogen equivalent is number of atoms of halogen absorbed per mole of compound.

These authors noted, however, that, although castor oil yielded the expected iodine number of 84.0 with the usual Hanus reagent, the addition of mercuric acetate resulted in an iodine number of 90.7. They attributed this anomaly to ricinoleic glycerides, since a similar result was obtained with petroleum ether–extracted castor oil acids.

In connection with other work, the authors had prepared pure methyl esters of ricinoleic and ricinelaidic acids (the *cis* and *trans* isomers of 12-hydroxy-9,10-octadecenoic acid), and had independently observed an analogous effect while determining their iodine numbers by the Wijs mercuric acetate technique. They further observed that Wijs reagent alone adds quantitatively to methyl ricinoleate and methyl ricinelaidate. Despite the absence of mercuric acetate catalyst, the reaction occurred with surprising speed, being essentially complete in less than 1 minute. To determine whether Wijs reagent was unique in its ability to add rapidly to these unsaturated compounds, a standard solution of bromine in glacial acetic acid (*5*) was also tested. It was found to react with equivalent speed.

In Table I, data are given which indicate the rate of reaction of methyl ricinoleate and methyl ricinelaidate with Wijs and bromine reagents. Aliquot portions from a standard solution of each of the esters in glacial acetic acid were used to obtain samples of equal weight. The molar concentration listed in the tables are the values calculated for the solutions obtained by mixing the reagents.

To determine the effect of mercuric acetate on the course of the reaction, the halogenating agent was mixed with the esters and the mixture then treated with mercuric acetate solution. The time was recorded from the addition of the latter. Examination of the data from these experiments (see Table II) demonstrates that the addition of mercuric acetate to solutions of dihalogenated ricinoleates or ricinelaidates in Wijs or bromine in acetic acid solutions results in a further utilization of the excess free halogen present.

Since the ricinoleates and ricinelaidates differ from the normal unsaturated fatty acids only by the possession of the 12-hydroxyl group, it is reasonable to conclude that the further utilization of halogens in the presence of mercuric acetate takes place at the hydroxyl group, probably involving an oxidation of the secondary alcohol group, or a substitution activated by the hydroxyl group.

In another series of experiments, three samples of methyl ricinoleate were allowed to react with Wijs solution for 30 minutes. One of the samples was titrated, and the iodine value was determined to be 82.1. To the other two samples, mercuric acetate was added, and at the end of 3 more minutes, the iodine value had risen to 88.8, and finally after 15 minutes, to 100.4.

Table II. Effect of 0.0147 *M* Mercuric Acetate on Iodine Values

Time, Min.	Halogen Equivalent	Iodine Value
A. 0.01968 *M* Methyl Ricinoleate, 0.05257 *M* Wijs		
1.0	2.054	83.44
3.0	2.188	88.91
3.0	2.187	88.82
9.0	2.368	96.21
15.0	2.470	100.31
15.0	2.473	100.45
B. 0.01847 *M* Methyl Ricinelaidate, 0.05257 *M* Wijs		
1.0	2.051	83.30
3.0	2.116	85.97
10.0	2.193	89.08
15.0	2.206	89.61
C. 0.01968 *M* Methyl Ricinoleate, 0.05644 *M* Bromine		
1.0	2.032	82.53
3.0	2.071	84.12
10.0	2.123	86.22
20.0	2.172	88.23
60.0	2.318	94.16
D. 0.02991 *M* Methyl *O*-Propionylricinoleate, 0.05246 *M* Wijs		
1.0	1.989	68.52
3.0	1.983	68.29
15.0	1.993	68.65

To prove conclusively that the excessive utilization of the halogen reagents was due to the free hydroxyl group, methyl *O*-propionylricinoleate was prepared and examined as above. The behavior of this ester was entirely normal as shown in Table II, thus demonstrating that the anomalous behavior of the ricinoleate and ricinelaidate is due to its free hydroxyl group.

From a practical point of view, the results of Wijs mercuric

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acetate iodine number determinations should be critically examined if the presence of free hydroxyl groups is suspected.

EXPERIMENTAL

PREPARATION OF METHYL RICINOLEATE (*3*). Castor oil was converted to castor oil methyl esters by saponification, isolation of the acids, and esterification with 2 to 4% sulfuric acid in absolute methanol. The esters (650 grams) were fractionally distilled through a 60-cm. (24-inch) Vigreux column, and methyl ricinoleate was collected at 157° to 158° C. at 1-mm. pressure. The yield was 474 grams of material having the following constants: $n_D^{30} = 1.4596$, iodine value, 82.0 (theory, 81.2).

PREPARATION OF METHYL RICINELAIDATE (*3*). From 2000 grams of castor oil, 530 grams of crude ricinelaidic acid were obtained by elaidinization of the acids with oxides of nitrogen (*3*). The acids were converted to the methyl esters by refluxing with 2 to 4% sulfuric acid in absolute methanol and separated from nonvolatile material by distillation from a Claisen flask at 1-mm. pressure. There was obtained 465 grams of impure product. This was then fractionally distilled as above, and the main fraction collected, b.p. 181° at 2 mm., and recrystallized twice from a mixture of Skellysolve F and diethyl ether. The yield was 290 grams of pure methyl ricinelaidate, m.p. 30° to 31° C., $n_D^{30} = 1.4582$, iodine value, 81.5 (theory, 81.2).

PREPARATION OF METHYL *O*-PROPIONYLRICINOLEATE (*6*). Castor oil methyl esters, prepared as above, were heated rapidly and briefly to 180° C. with two-thirds their weight of propionic anhydride. The mixture was fractionally distilled to obtain the pure ester which had the following constants: b.p. 186° at 1 mm., $n_D^{30} = 1.4510$, saponification equivalent, 185.6 (theory, 184.3), acid value, 0.0, and iodine value, 68.5 (theory, 68.9).

PROCEDURE. Automatic pipets were used to obtain aliquots of all standard solutions. An aliquot portion (10.96 ml.) of the compound and 24.98 ml. of the halogen reagent were mixed and allowed to react for the stated time interval, and the excess reagent was then titrated to obtain the results given in Table I. To determine the effect of mercuric acetate shown in Table II, the ester and halogen reagent were mixed, and immediately treated with 10.0 ml. of 2.5% mercuric acetate in glacial acetic acid, and allowed to react for the intervals recorded, beginning with the addition of mercuric acetate. Blank experiments, without the presence of the unsaturated compound, were performed in all the series to eliminate corrections necessitated by the presence of small amounts of oxidizable materials in the mercuric acetate solution.

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Improved Device for Decomposition of Grease

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IN THE laboratories of the Rock Island Arsenal, where the number of grease samples to be analyzed and the time available are determining factors, the standard method of the American Society for Testing Materials (*1*) for decomposing the soap in the grease was too time-consuming when a 30-gram sample was used, the time varying from 20 minutes with a light grease to 2 hours with some of the heavier greases. This method of soap decomposition consists essentially of shaking 8 to 30 grams of the sample, depending on the consistency of the grease, in a separatory funnel at room temperature with petroleum ether and 10% hydrochloric acid.

Since the "boiling method" is used in many grease testing laboratories, its suitability for the Arsenal needs was looked into. Briefly, this method consists in placing about 30 grams of grease in a 400-ml. beaker, adding about 200 ml. of 10% hydrochloric acid solution, and then heating the mixture to the boiling point of the hydrochloric acid solution to accelerate the decomposition. It was found that the time required to break down the grease could be reduced to from 5 to 10 minutes for the average grade to 20 minutes for the heavier grades. This method does not require the undivided attention of the analyst; however, unless the mixture is constantly stirred, steam has a tendency to build up pressure beneath the floating grease layer. In the case of the heavier grades of greases especially, this steam pressure may cause violent bumping with subsequent loss of material due to splattering.

To overcome this, several procedures were tried but only two proved effective. The first, which was very effective but cumbersome, consists in placing a slow-speed motor on a rack over the hot plate and stirring the mixture as it boils. The second procedure is the percolator method.

When the grease percolator, as shown in Figure 1, is placed in the beaker with the grease sample and the hydrochloric acid solution, the steam is provided with an outlet, thereby preventing excessive bumping due to the pressure buildup. The percolator serves a twofold purpose. Primarily, it serves to prevent violent bumping with its subsequent loss of sample and, secondly, it is used to agitate the grease, thus accelerating the decomposition by exposing more surface to the acid solution. This secondary effect is accomplished by means of the twist in the steam tube (see diagram). When the mixture reaches the boiling point of the hydrochloric acid solution, steam and hydrochloric acid solution "boil up" through the percolator out of the tube and are forced back into the surface of the grease layer by their own pressure. By placing the exit tube at an angle, the steam and hydrochloric acid solution which spurt out with some force tend to agitate the grease layer, causing it to revolve on the surface of the hydrochloric acid solution.

The percolator can be readily made in the laboratory from an ordinary 2.5-inch diameter 60-degree angle soft-glass funnel. Two or more venting grooves are bent in the lip of the funnel. The stem is then bent in the manner shown by the diagram.

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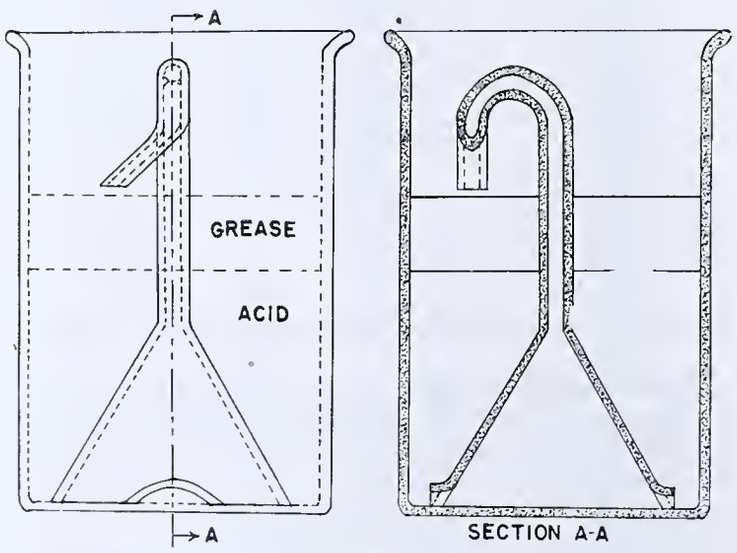

Figure 1

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Distillation Trap for Determining Moisture in Relatively Dry Materials

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A COMMON method for rapid determination of moisture is distillation of the material with a solvent immiscible with water and collection of the water in a suitable trap where its volume may be measured. Several types of distillation traps are described in the literature (*1, 3–10, 12*), the earliest being that of Bidwell and Sterling. These traps have been designed to use solvents with densities both heavier and lighter than water. Liquids heavier than water have several advantages, other than their usual nonflammability. Since most samples float on the heavy solvent, scorching on the bottom of the flask and excessive bumping are eliminated. The vapor and condensate are at all times in the path of continuous flow, with no stagnant pockets in the condensed solvent or water where individual droplets form and remain isolated.

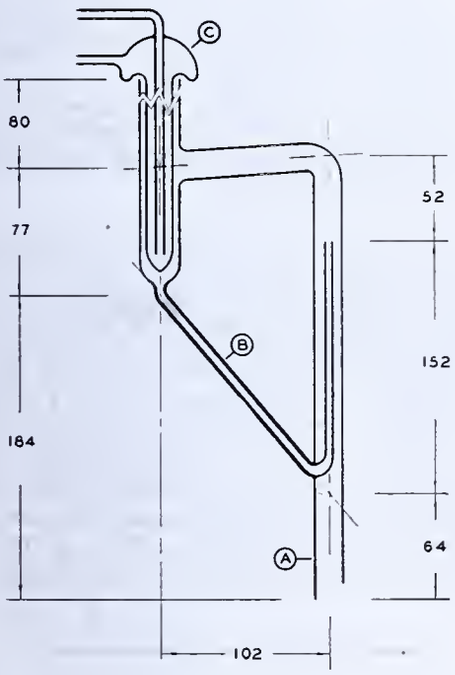

Figure 1. Diagram of Trap

Dimensions in mm.

The usual trap is graduated in 0.1 ml., and the volume of water collected may be estimated to 0.01 or 0.02 ml. For products of low moisture content, a large sample must be taken to obtain the usual 5 or 10 ml. of water. Many products are too bulky for an adequate sample weight, and in many instances only small quantities are available for analysis. For these reasons a trap designed particularly for small amounts of moisture has been desired. Attempts to modify existing heavy solvent traps by decreasing the diameter of the usual graduated tube to secure smaller unit volumes were not satisfactory because the solvent did not fall dropwise through the water layer, and the water was swept out of the trap. As a result, a new type of tilt trap (Figure 1) using a heavy solvent was designed to allow precise measurement of small volumes of water.

DESCRIPTION OF TRAP

Tube *A* (17 mm. in diameter) is connected to the boiling flask with a cork or ground-glass joint. Vapors from the boiling flask pass through *A* into the 25-mm. diameter section of the trap containing an immersion condenser, *C*, the shoulder of which rests loosely on the top of the trap, and positions the tip of the condenser near the graduated arm, *B*. It is important that the condenser be immersed in the condensed water and solvent. *B* was made of a 1-ml. Pyrex pipet with 0.01-ml. divisions. The portion of the return line inside tube *A* was made of 6-mm. tubing. The height of the open end of this tube was adjusted to cause the condensed water layer to remain in the 25-mm. diameter tube above the constricted section. When the trap is tilted to bring *B* into a vertical position, the water layer falls into this graduated tube, where the water volume can be read directly to 0.01 ml. and estimated to 0.001 ml. As the zero graduation mark will not necessarily coincide with a meniscus, the volume is determined by taking the difference between the meniscus readings. The trap was designed for use with perchloroethylene $\left(d = 1.63\frac{15}{4}\right)$; other solvents may be used if their density is near that of perchloroethylene.

OPERATION OF TRAP

The sample is weighed into a 250-ml. boiling flask containing 150 ml. of perchloroethylene, the trap is connected, and cold water is circulated through the condenser. It is important to fill the return line of the trap with solvent before distillation starts, in order to prevent water from entering the small diameter tubing. The content of the flask is quickly brought to boiling, and maintained at a vigorous boil. It is necessary to have a large flow of condensed solvent through the return line. If the flow is small, the solvent in the portion of the return line inside tube *A* may boil and the water layer may fall into the return line and completely upset the correct operation of the trap.

Small droplets that sometimes remain on the condenser and glass surface of the trap at the completion of the distillation will unite with the condensed liquids if the condenser is lifted and touched with a small rod (1 mm. in diameter) wetted with Tergitol 4 or 7. An excess of wetting agent will make it difficult to read the meniscus between the solvent and water, because of formation of a cloudy layer. When the condenser is replaced and the contents of the trap are gently swished, the glass surfaces will drain. Since the condenser maintains the water layer and condensed solvent near room temperature, the water volume may be read as soon as boiling has ceased, thereby shortening by several minutes the total time of the determination.

The trap was tested for water recovery by placing 0.50 ml. of water in the boiling flask with 150 ml. of perchloroethylene and boiling for 30 minutes. The water recovery was 0.48 ml. Cause for the discrepancy of 0.02 ml. was not determined, but is probably water held on the surfaces of the trap and condenser. When determining the moisture content of a foodstuff, the incomplete recovery of water may be neglected, because decomposition of the sample at the high temperature of the boiling solvent requires an arbitrary adjustment of the total distillation time to produce agreement between the distillation method and a reference method. For whole egg powder, with perchloroethylene as the solvent, a distillation time of 25 minutes was found to give results comparable to those obtained by the A.O.A.C. (*2*) and the faster high-vacuum methods (*11*).

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Manometric System for Volumetric Gas Analyzers

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THE use of nonscientific personnel in operation of the so-called precision model volumetric gas analyzer has presented many difficulties in this laboratory, which has to analyze several hundred gas samples a week. The two most troublesome are blowing out or sucking in of manometer fluid by the operator, and the development of a leak in the middle of an analysis.

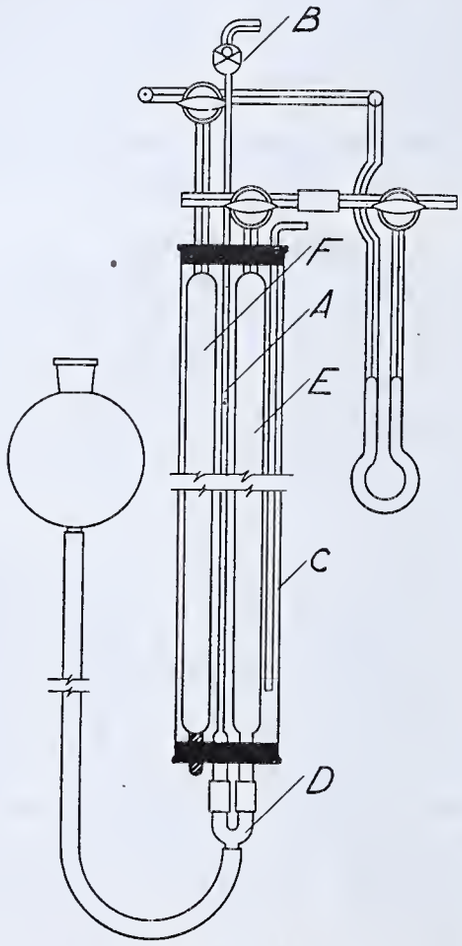

Figure 1. Improved Manometric System as Installed in a Regular Gas-Measuring Unit

The first difficulty is frequent with beginners but occasionally happens during fast operation to the most experienced gas analyst. With standard apparatus this is usually due to difficulty in obtaining accurate equalization of the mercury levels in the leveling bulb and the gas buret, particularly in the lower portions of the system, or failure to close the manometer stopcock before starting the flow of gas. The second difficulty is usually due to excess positive or negative pressure in the system.

These difficulties and the time lost in dismantling, cleaning, and reassembling apparatus, refilling the manometer, and repeating analyses, prompted the design of the system described below.

Nearly all commercial models of volumetric gas analyzers contain one ordinary U-type manometer with one arm attached to the compensating tube and the other to the manifold or to the buret itself. The system described here consists of two manometers, one for rough preliminary equalizing of the gas pressure and the second for sensitive and accurate final pressure balance. For the latter, the manometer contained in the original apparatus serves very well when filled with a slightly acidified aqueous solution.

For the first manometer, a design was worked out which roughly equalizes the gas pressure before opening the sensitive manometer to the system, gives a continuous visual indication of the pressure within the system, and compensates partially any abnormal pressure or vacuum developed in the system during the analysis. This manometer is built into the water jacket and makes use of the gas buret for one arm of the U. The other arm is a small-diameter glass tube which connects to the gas buret at the base by means of a small glass Y.

The system is shown in Figure 1 as it was built into the regular buret manometer-compensator assembly.

The left arm, *A*, of the built-in manometer is constructed of 6- and 8-mm. tubing, the base being 8-mm. tubing to correspond with the gas buret outlet. The main body of the arm is of 6-mm. tubing and has a small ground-glass, ball check valve, *B*, sealed to the top. The over-all length of this tube for a 77.5-cm. (31-inch) water jacket, *C*, is 100 cm. (40 inches) and it contains approximately 12 ml. of mercury when completely filled. The connecting Y-tube at the base is of 8-mm. tubing and has 2.5-cm. (1-inch) arms to facilitate good seals with the rubber connecting tubing.

The other parts of the assembly are self-explanatory. The compensator tube, *F*, was placed in the abnormal position shown, for clarity on the two-dimensional drawing, and normally occurs directly behind the gas buret, *E*.

This manometer system (1) eliminates the guesswork involved in equalizing the mercury levels before opening the sensitive manometer to the system, thus preventing one of the causes of blowout or suckin of manometer fluid; (2) it helps to compensate for any abnormal pressure or vacuum which may be exerted on the system, thereby decreasing leaks; and (3) it gives the analyst a continuous visual indication of the pressure within the system throughout the entire analysis. All these factors contribute toward faster and more consistently reliable analyses. The system makes cleaning and reassembling of the buret unit somewhat more difficult; however, used on several analyzers in this laboratory for more than a year, it has helped to prevent errors.

Lubricant Reservoir for Stirrer Shaft

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IN ORDER to avoid liquid-sealed stirrers, the author has been using stirrers of the type described by Fieser (*1*). The stirrer bearing is equipped with a 2-cm. section of rubber tubing which projects above the end of the bearing and makes a tight fit on the stirrer shaft. This rubber bearing requires frequent lubrication to prevent heating and consequent impairment of the seal.

A very satisfactory lubricant reservoir can be made by cutting off the closed end of a medicine dropper bulb and fitting the decapitated bulb over the rubber bearing. This device has been found serviceable over long periods of time, requiring only a few drops of glycerol or heavy mineral oil once a day.

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Experimental Dryer for Pre-Pilot Plant Studies

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IN DESIGNING pilot-plant equipment with which to study the factors involved in large-scale processing of various products developed in the laboratory, experimental drying apparatus is frequently needed capable of determining the drying characteristics of different materials under widely varying conditions and of yielding data translatable to larger scale operations. Such a machine should require a small quantity of material.

In recent years, drying techniques have been aimed at achieving the most rapid rate of drying possible without introducing deleterious effects on the product and consistent with the most economical methods for handling the material. This has been accomplished in many cases by employing a through-air circulation method of drying, by which heated air is forced at high velocity through a porous bed of the material supported on a perforated-metal or screen surface. An experimental unit which meets these requirements is here described.

EQUIPMENT

Figure 1 shows a general layout of the unit. It consists essentially of a centrifugal blower, *A*, 7.5 inches in diameter with a V-belt drive from a 0.5-horsepower motor; a drying cabinet, *B;* a torsion balance, *C*, reading to 0.2 gram; an automatic wet and dry-bulb recorder-controller, *D*, having throttling control and automatic reset and equipped with a chart reading to 300° F.; an air-conditioning cabinet having a 2-row finned-tube steam coil with 39 square feet of heating surface; plus ductwork, control valves, and accessories. The entire unit is constructed of sheet metal covered with 2 inches of 85% magnesia insulation.

For supporting the material several stainless steel pans are provided, each 1 square foot in area. The bottoms are constructed of perforated metal or wire screen having apertures of various sizes for different kinds of material. Pan inserts are also provided to reduce the area of the bottom or increase the height of the walls. Nichrome wires suspended from an aluminum rod and clamped to the drying pan at its four corners pass through the roof of the drying cabinet (Figure 2). The holes in the roof are sealed with rubber stoppers.

The inside cross-sectional area of the drying cabinet is 4 square feet and the distance from the pan shelf to the roof is 2 feet. Thus when the air enters the drying cabinet, its velocity is reduced to a very low value. When the pan is elevated by the wires approximately 10 inches above the shelf and suspended from the balance on the roof, accurate weighings can be made very rapidly without shutting off the air supply and upsetting the control conditions. A further advantage of this plenum chamber above the drying pan is that it produces uniformity of air flow through the 1 square foot of drying surface of the pan without baffles or vanes.

Starting at the blower, the air flows through the upper duct to the drying cabinet. At location 1, Figure 1, is a manually operated volume-control damper, by means of which the total flow of air is regulated. The air next passes through an orifice nozzle, 2. Centrally located in the nozzle is a Pitot tube, which is connected to the upper inclined manometer on the cabinet and gives a reading of velocity head in inches of water; from this and from the temperature the volume-flow of the air is calculated. The air now enters the drying cabinet and passes down through the material in the pan. The pan rests on a shelf which forms an orifice in the drying cabinet. Static pressure openings above and below the shelf and connected to the lower inclined manometer indicate the loss in pressure as the air passes through the material and the pan. This information is vital to the design of a blower for larger equipment.

Mounted just above and behind the drying pan are the dry and wet bulbs of the air-operated temperature recorder-controller (Figure 2), by which the temperature and humidity of the air blowing on the wet material are controlled. A slight error in the wet-bulb temperature of the drying air is introduced here, since the evaporation from the wick is included in the air passing through the pan. Also entering the drying cabinet just above the pan is an iron-constantan thermocouple, connected to a potentiometer. The thermocouple lead wires are coiled, so that when the point is injected into the wet material, the pan can be raised and lowered for weighing and the additional tare due to the thermocouple will be practically constant.

As the air leaves the bottom of the pan, it passes over the sensitive portion of an industrial thermometer and then enters the return duct. Here the amount of spent air vented at 4 is automatically controlled by damper, 3, operated by a diaphragm motor which functions from the wet bulb of the controller. An equal amount of fresh air enters at 5. The recirculated air plus the fresh air then enters the conditioning cabinet.

In the conditioning cabinet the air first passes through the steam heater. The supply of steam is automatically controlled by a diaphragm valve which functions from the dry bulb of the instrument. If necessary, the heated air is then humidified by spraying steam or a fine mist of water through nozzles facing against the path of air flow. The quantity sprayed is automatically controlled by a diaphragm valve which functions from the wet bulb of the instrument. Either water or steam can be supplied to the humidifying control valve, the choice being determined by the wet-bulb temperature required.

[1] Present address, National Drying Machinery Co., Philadelphia, Pa.

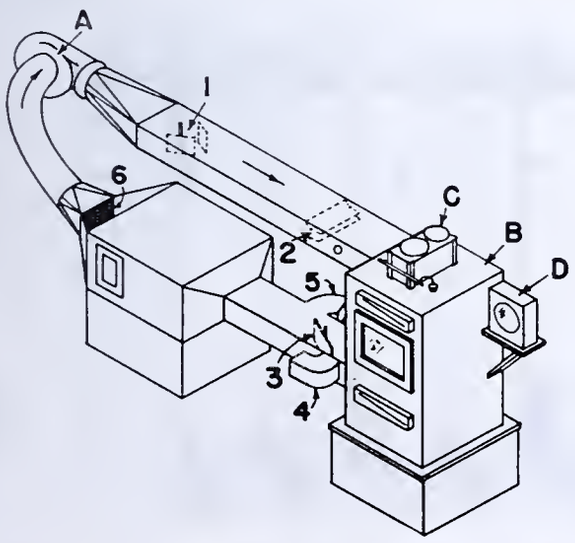


Figure 1. Dryer

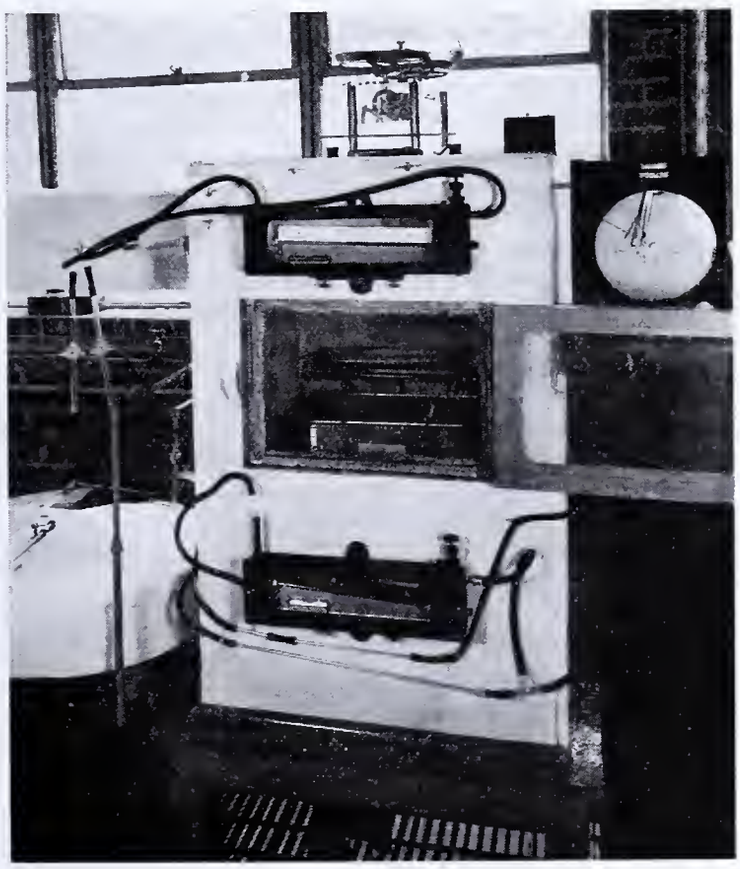
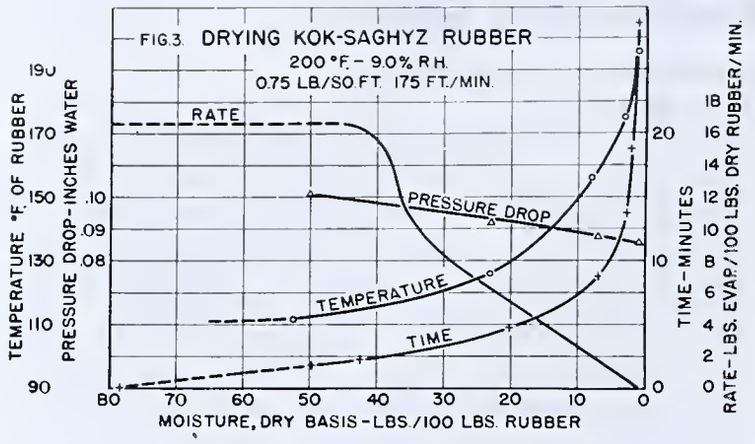


Figure 2



The conditioned air finally passes through a copper diffusing screen, 6, which eliminates any droplets of water. If the humidifying nozzles were located in the duct leading to the heater, the diffusing screen would be unnecessary. The air now returns to the blower for another cycle.

EXAMPLE OF USE

The utility of such an experimental dryer was demonstrated during the development of a pilot-plant process for extracting rubber from the Russian dandelion.

The purified rubber as extracted from the root was in the form of small discrete particles which floated in water. This material was first dewatered on a centrifuge, then spread into a drying pan having a perforated metal bottom. Studies were made to determine the optimum air temperature, velocity, depth of loading, etc. Samples from each test were checked by compounding the rubber and subjecting it to physical tests. The type of drying data obtained from a single test is illustrated in Figure 3. Air at 200° F. and 9% relative humidity was circulated at a rate of 175 c.f.m. per square foot of pan area; the tray was loaded with 0.75 pound (dry weight) of rubber per square foot.

It was demonstrated that such a heat-sensitive material could be dried very rapidly at an elevated temperature with better results and more economically than by a conventional method for drying rubber, such as vacuum drying. The data obtained made possible the operation of a pilot-plant dryer under conditions which yielded the best quality of dried rubber.

Pycnometer Holder

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SINCE the publication by Lipkin, Davison, Harvey, and Kurtz (*1*) of a new design of pycnometer especially suited to the precise determination of the densities of volatile liquids, routine use of this instrument has indicated the need for a multiple holder assembly with which to support two or more pycnometers at one time in a glass jar thermostat. A simple and satisfactory type of holder which has been in use in this laboratory for several years is illustrated and described herewith.

Figure 1 shows the structural details of the holder proper.

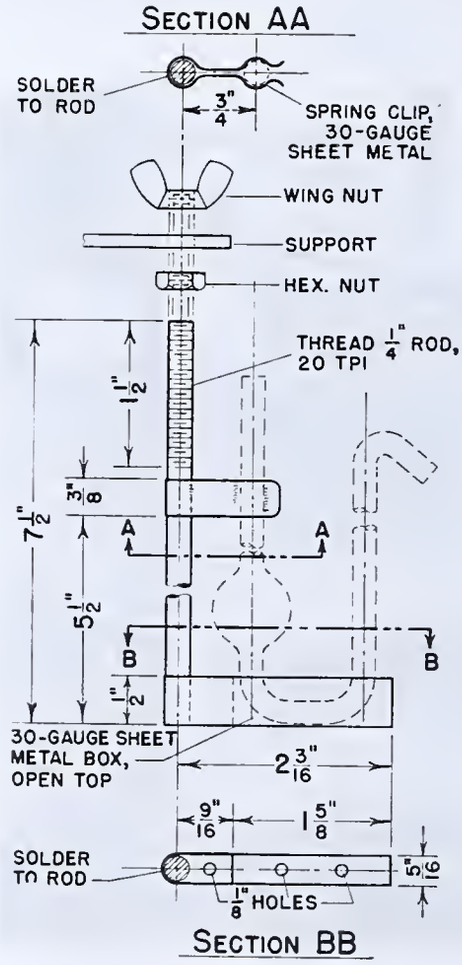

Figure 1. Pycnometer Holder Detail

It may be made of brass or any other available metal which can be hard- or soft-soldered and will not corrode in the thermostat liquid.

Figure 2 illustrates a convenient mounting for suspending the holders in the thermostat. It consists of a brass bar 0.125 inch thick, 1 inch wide, and 12 inches long with seven $^{9}/_{32}$ inch holes drilled 1.5 inches apart to accommodate the threaded ends of the holders. Two nuts support each holder and permit regulation of the depth of immersion of the pycnometers. A total of six holders with pycnometers may be conveniently suspended in a 12-inch diameter jar with this mounting. The ends of the mounting as illustrated are drilled to fit over posts clamped to the sides of the thermostat. However, the posts need not be used, the bar simply being allowed to rest on the edges of the jar. Individual mountings for each holder which can be hooked or clamped to the edge of the jar may be used if desired.

Wing nuts provide a convenient means for rotating the holders to the best position for reading the levels of the menisci. Pycnometers may be placed in or removed from the thermostat without removing the assembly from the bath or disturbing other pycnometers mounted in it.

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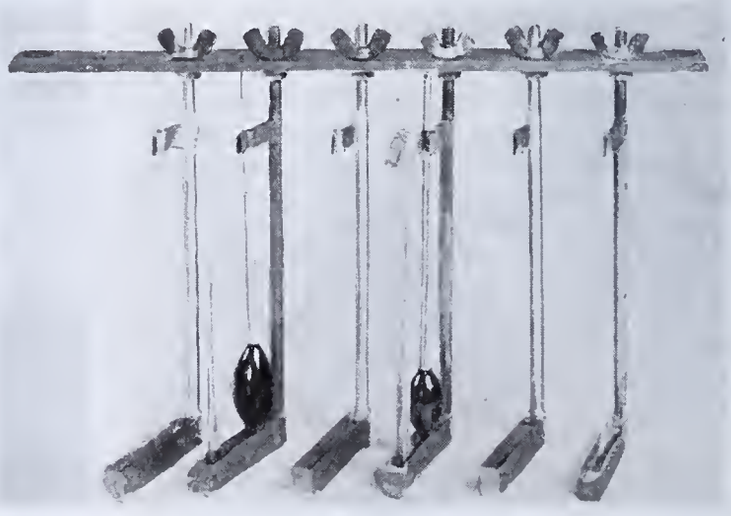

Figure 2. Multiple Holder Assembly

A Combination Glass Soxhlet Extractor and Vapor Degreaser for General Laboratory Use

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IN CONNECTION with investigations of certain types of insulating materials, such as capacitor dielectrics (impregnated paper, mica, ceramics, and plastics), extraction procedures and suitable techniques for proper cleansing of instruments and test specimens are of basic importance. The usual type of glass Soxhlet extractor and various modifications (the literature is voluminous in this connection), including metal and earthenware types designed particularly for use in biochemical and nutrition laboratories (*1*, *2*, *3*), have this in common, that the condenser is made the top (cover) section of the extraction compartment or is adapted to it. This necessitates removal of the condenser when introducing and withdrawing the parts to be treated. Frequently, operations of the sort referred to above must be performed with dispatch, requiring minimum manipulation. Furthermore, it is often desirable to treat instruments and complete or partial test assemblies whose geometric shapes, particularly as regards their length, are such that commercially available equipment will not accommodate them.

To overcome these limitations a combination extractor and degreaser has been designed and constructed in which the water condensers are integral with the liquid (extraction) and vapor (degreasing) compartments, so that there is direct access to these compartments without manipulation of the apparatus.

As shown in Figure 1, the apparatus consists of two parts: a solvent container (flask) and an extractor and degreaser unit, both of Pyrex. The flask is fitted with a female section of a $\frac{45}{50}$ ground-glass joint. The male section of this joint is attached to the lower end of the extractor and degreaser unit. Vapor ducts leading from the flask to the extraction and degreaser unit, as well as the degreaser compartment itself, are wrapped with asbestos tape to reduce condensation on the walls of these components. Approximate over-all dimensions of the apparatus are 62.5 cm. (25 inches) high × 20 cm. (8 inches) wide × 16.25 cm. (6.5 inches) deep. The flask is charged with a volume (1200 cc.) of solvent approximately three times the minimum required to operate the extractor. The heating is controlled to provide distillation at a mild rate. Under these conditions the data in Table I have been obtained on the rate of solvent loss by evaporation.

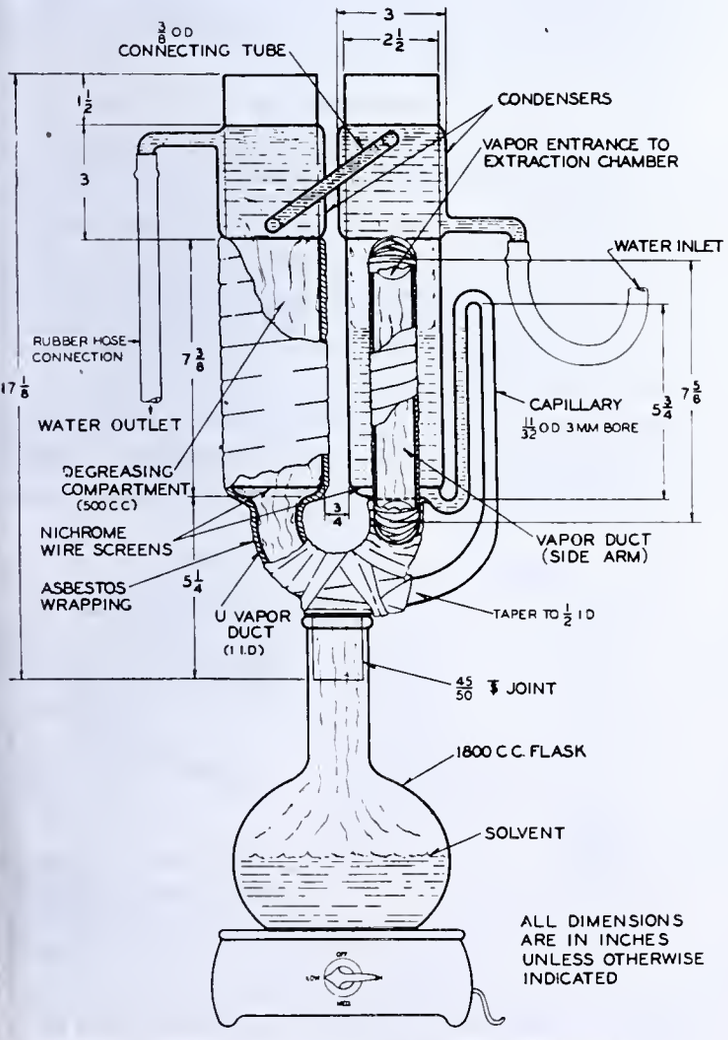

Figure 1. Soxhlet Extractor and Vapor Degreaser

Table I. Performance of Apparatus

Solvent	Rate of Evaporation Loss *Cc./hour*	Extraction Cycle *Min.*
Benzene	5	45
Acetone	10	40
Ether	24	25
Trichloroethylene (Permachlor)	2	60

Some reduction in the consumption rate may be effected by providing caps for the tops of the compartments. However, experience has shown that without caps the loss in most cases is sufficiently low, so that the equipment is well adapted to protracted extraction treatments. Virtually all the vapor condenses on the lower third of the condenser surface. Additional solvent may be introduced through the degreaser compartment to replace solvent lost during operation. Because of the open-top feature, the equipment should be located under a hood or where the escaping vapor can be readily removed from the working area.

Without interrupting the boiling and condensation of the solvent, samples to be treated are inserted and withdrawn through the wide cylindrical mouth at the top of each compartment. The vapor phase chamber is distinct from the liquid extraction compartment, thus permitting vapor cleansing of laboratory appliances without contaminating test specimens in the extraction section. Thorough cleansing is often expedited by subjecting the work to a short treatment in the liquid phase followed by a dip in the vapor to remove last traces of contamination.

Because of its flexibility, this apparatus has served well as a general laboratory utility. The equipment may be constructed of metal or part metal, part glass. (In a modification of the all-glass unit the condensers have been constructed of metal in such a manner that the cooling liquid is in direct contact with the glass surface of the compartments. These condenser shells are firmly secured in proper position on the extraction and degreasing columns.) A proportionate increase or decrease in the dimensions of the component parts (with the exception of the capillary) should provide a unit which will satisfy specific requuirements.

ACKNOWLEDGMENT

The author wishes to acknowledge suggestions as to design details given by H. W. Weinhart, his colleague at these laboratories, who also supervised the construction of the apparatus.

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Reaction Vessel for Maintaining Constant pH by Continuous Electrometric Titration during Sodium Amalgam Reductions

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A NUMBER of chemical reactions occur at definite optimum pH but liberate substances which alter the pH. In order to maintain the desired pH it is therefore necessary to add controlled amounts of acid or base to the system during the course of the reaction. Such control has been accomplished by use of indicators, buffers, or electrometric instruments. The first method is not practical where the reaction medium is turbid or colored, or the pH of the solution is changing rapidly; the second is limited by questions of buffer capacity and the isolation of the product.

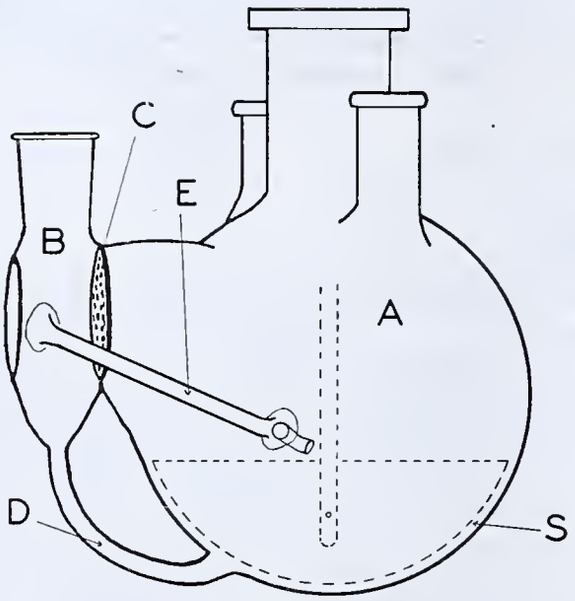

Figure 1. Reaction Vessel

Electrometric control can be maintained in large-scale reactions by the use of automatic potentiometric equipment. On a smaller scale it is possible to use an external centrifugal pump and electrode chamber with manual control of the addition of acid or base required to hold the pH constant, but this system requires fairly large volumes of liquid and may result in corrosion in the pump chamber. A number of micro and semimicro control techniques have been devised, but these methods are not readily adapted to the usual laboratory preparation (*1*).

A simple, all-glass vessel is needed to accomplish this purpose without the use of external circulating pumps and expensive automatic control instruments—i.e., one suited for laboratory use. The authors have devised such a vessel for use in studies of the sodium amalgam reduction of aldonolactones to aldoses where the reduction was studied at several pH ranges. With slight modification this vessel may be employed for many other types of reactions where pH control is necessary.

APPARATUS

The reaction vessel (Figure 1) consists of a 1-liter, three-necked Pyrex flask, *A*, with a small electrode chamber, *B*, sealed on just above the middle of the flask. Larger or smaller flasks may be used just as readily. A stainless-steel stirrer, *S*, having a hinged blade, which is a circular segment of the cross section of the flask and fitting to within 1 to 2 mm. of the bottom, furnishes the pumping action (*2*). The stirring motor should be both fast and powerful. A motor controlled by a centrifugal governor is preferable to one with a rheostat control.

The flask and the electrode chamber are separated by a perforated glass disk, *C*, 3 cm. in diameter, containing a large number of 1- to 2-mm. holes. The perforations serve to diminish the impact of the solution against the electrodes, especially when mercury is used in the reaction mixture. The neck of the electrode chamber is large enough to accommodate a No. 6 rubber stopper. The main portion of the electrode chamber is a flat cylinder 2.5 cm. deep on its horizontal axis, one base of which is the perforated disk. A conical taper, 2 cm. deep, connects the bottom side of this cylinder to the lower side of the flask at a point vertically about 1 cm. from the bottom of the flask by means of an 8-mm. glass tube, *D*, which has a slight U-bend. This tube serves as the main path for the return of the mercury from the electrode chamber to the flask. Another glass tube, *E*, connects the center of the electrode chamber to the lower third of the flask. This tube is joined to the flask by an inner seal just above the point where the stirrer blade moves along the wall of the flask and opens within the flask as a spout which points in the direction of movement of the blade along the wall. The tube acts as a siphon and keeps constant the level of the aqueous solution in the chamber; it also provides for a rapid change of the liquid in the electrode chamber.

The pH changes occurring in the electrode chamber can be most accurately followed when a direct-reading pH meter is employed, but the ballistic type of instrument may be used almost as readily. The electrodes may be constructed to fit the dimensions of the cell, or any of the long electrodes furnished commercially can be adapted to fit the cell. An agar–potassium chloride bridge was found to be superior to the capillary glass type, but a commercial calomel cell with a flexible rubber tip containing a pinhole was satisfactory.

The electrodes are inserted in a two-holed rubber stopper and are adjusted to fit into the lower portion of the electrode chamber without touching the sides. A 50-ml. calibrated buret is placed in one side neck and the other neck is available for measuring the temperature and adding reagents.

OPERATION

A 10- to 15-ml. pool of clean mercury is placed in the flask and the stainless-steel stirrer is fitted as close to the bottom as possible. The bearing for the stirrer may be simply a glass tube with a rubber–mineral oil seal. The speed of the stirrer is considered properly adjusted when all the mercury is thrown against the side of the flask and no large globules remain at the bottom when the stirrer is operated. The reagents are added together with 200 ml. of water and the speed of the stirrer is regulated to force the solution and the stream of mercury into the electrode chamber at a level sufficient to keep the electrodes covered. The electrode assembly is then inserted into the neck of the electrode chamber.

The stirrer and the whirling mercury produce an oscillating, pumping action whereby the liquid and the mercury are forced into the chamber and back through the return tubes. Mercury collects in the U-portion of tube *D* during one phase of the action,

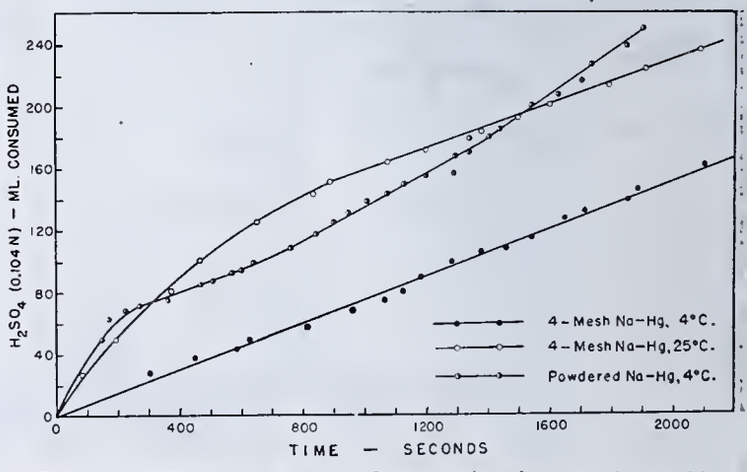

Figure 2. Decomposition of Sodium Amalgam in 0.104 *N* Sulfuric Acid

[1] Present address, Schering Corporation, Bloomfield, N. J.

and when the oscillating solution moves away from the opening, a stream of mercury and liquid pours back into the flask. The whirling solution creates a suction on the siphon tube, *E*, and keeps the liquid in the electrode chamber at a constant level. The stream of mercury mixes the contents of the chamber, so that the pH readings are representative of the whole sample.

The pH control is effected as follows: One hand is used to operate the buret which contains the standard acid or base while the other hand taps the electrometer key. Thus, if base is liberated during the experiment and the desired range is 3.5 to 4.5, the dial of the pH meter is set at pH 4 and standard acid is added at a rate which causes minimum deflection of the needle. With a little experience the operator can maintain the pH of the reaction mixture within 0.5 to 1.0 pH units in a continuously changing system.

EXAMPLE. The precision of the method can be demonstrated by a study made on the rate of decomposition of 25-gram samples of 2.5% sodium amalgams of different particle size and at various temperatures in contact with an aqueous phase maintained in the pH range of 3.5 to 4.5. The deviation of the points from the smooth curve in Figure 2 may be taken as a measure of the degree of control attained. The entire reaction vessel may be submerged in a constant-temperature bath when it is desirable to control the temperature at which the reaction is allowed to proceed.

ACKNOWLEDGMENTS

The authors wish to thank Harold E. Zaugg of the Abbott Laboratories, North Chicago, Ill., for furnishing the stainless-steel stirrer and for helpful advice during the course of the work.

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Estimation of Iodine Color of Starches and Starch Fractions

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Starch and Dextrose Division, Northern Regional Research Laboratory, Peoria, Ill.

STARCHES and starch fractions are usually characterized in part through the color produced by addition of iodine to their solutions. Exact comparison of these solutions can be made only by means of the spectrophotometer (*1*, *3*, *4*, *5*, *9*), but in many instances, and for routine testing, visual estimation of color is useful. Heretofore, however, no standard method for comparing starch-iodine colors has been employed, and, in most cases, authors have failed to record the procedure used in making the color test. Since the color with iodine of some starches and starch fractions changes with the concentration of the starch solutions and with the amount of iodine added, many starch-iodine colors recorded in the literature without accompanying description of the test method are not suitable for comparative work.

In order to facilitate and standardize the designation of color of starch-iodine solutions, the following method has been devised for visual comparison of these solutions. The method gives reproducible results and is sensitive to small differences in color. All colors are referred to standard color charts (*7*, *8*) and hence are capable of direct comparison in different laboratories

Color names have been chosen with a view to keeping the nomenclature as simple as possible. In general, comparisons have been made with standard colors or with common color names. It is recognized that the designation of color by name is inexact to the extent that most names may properly describe a number of colors within a given small range. Hence, for more exact designation of color, the more precise notation devised by Munsell (*7*) has also been used. The results obtained in applying the test method to several starches and starch fractions are shown in Table I.

The method is particularly useful in determining the purity of amylopectin fractions. The presence in amylopectin of amylose in quantities so low as to be scarcely evident on potentiometric iodine titration (*2*) is readily shown by the blue color produced on addition of 0.1 ml. of iodine to the starch dispersion, while more iodine up to 0.5 ml. brings out the typical amylopectin color. The persistence of the blue color and its influence on the final color may serve as a rough indication of the amount of amylose present. With appreciable amounts of amylose in the amylopectin, the blue color of the amylose-iodine complex masks the color of the amylopectin with iodine and prevents its visual detection. This method, therefore, does not serve to detect amylopectin in mixtures where amylose constitutes more than 6 to 8% of the total carbohydrate present. In the latter solutions the amylose-iodine complex usually precipitates.

Under the controlled conditions of test employed here, the color produced by iodine on waxy cornstarch is true purple and not red as has often been reported. The red (sometimes called reddish-brown) color frequently associated with waxy cornstarch is observed only in the presence of an excess of iodine and is in part due to the color of the free iodine in the solution. A number of intermediate stages of color can be observed (*6*) in waxy

Table I. Colors Produced by Iodine on 0.03% Dispersions of Starches, Starch Fractions, and Starch Derivatives

	Colors Observed with Various Amounts of 0.01 *N* Iodine on 10-Ml. Starch Dispersions			
	Color Comparison Made with Ridgeway Color Charts (*8*)		Color Notation from Munsell Color Charts (*7*)	
Starch Sample	0.10 ml. (2 drops)	0.50 ml. (10 drops)	0.10 ml. (2 drops)	0.50 ml. (10 drops)
Amylose	Spectrum blue	Deep blue precipitate	5PB 4/12	5PB 3/12
Whole cornstarch (disintegrated)	Spectrum blue	Deep blue precipitate	5PB 4/12	5PB 3/12
Corn amylopectin (not purified)[a]	Light spectrum blue (49b)[b]	Spectrum violet	5PB 5/10	3P 4/10
Corn amylopectin (cotton treated, *1*)[c]	Light blue-violet	Amethyst-violet to violet-purple	9PB 5/8	4P 3/10
Potato amylopectin[c]	Light purple (65b)	Magenta	10P 5/10	5RP 4/12
Waxy cornstarch (hand-pollinated)	Light amethyst-violet (61b)	True purple to magenta	5P 5/8	4RP 4/10
Waxy rice	Light pink (67f)	Reddish magenta (69')	5RP 7/8	7RP 4/12
Waxy barley starch	Light spectrum violet (59b)	Violet-purple	10P 5/10	10P 4/10
β-Amylase limit dextrin (cornstarch)	Spectrum blue	Spectrum violet	5PB 4/12	3P 3/10
Wheat dextrin (roasted 6 hours at 180° C.)	Light amethyst-violet (61b)	True purple	5P 5/8	4RP 4/10
Wheat dextrin (roasted 6 hours at 190° C.)	Pale pink (3'f)	Light jasper red to light coral red	8RP 7/8	4R 6/10

[a] Iodine absorption (*2*) 16 mg. per gram; approximate amylose content 8.0%.
[b] Numbers refer to plates whose color nomenclature has been simplified.
[c] Approximate amylose content 0%.

starch dispersions as the amount of iodine is increased toward an excess. Under identical conditions of test the waxy starches seem to differ in the color produced by iodine as one proceeds from type to type. Listed in order of decreasing red and increasing violet contents the starch-iodine dispersions may be arranged as follows: waxy rice, waxy corn, and waxy barley. To this series may be added in order potato amylopectin and corn amylopectin. This order coincides with the arrangement of Baldwin, Bear, and Rundle (*1*) who have suggested that it is also the order of increasing length of chain ends for the respective starch molecules.

METHOD

Thirty milligrams of starch are dispersed in 10 ml. of 1 *N* potassium hydroxide by allowing the mixture to stand with occasional shaking at 0° C. for 1 to 2 hours. For starch in the whole-granule state, 20 ml. of 0.5 *N* potassium hydroxide are used for the dispersion. The dispersion is neutralized with 1 *N* hydrochloric acid to a phenolphthalein end point and 1 drop of acid added in excess. The solution (pH 4 to 6) is then made up to 100-ml. volume, giving a starch concentration of 0.03%. By proceeding in this fashion, a constant amount of salt is introduced into each sample. Large amounts of salt, which produce changes in the iodine color, are to be avoided. To 10 ml. of the solution in a 16- by 150-mm. test tube is added 0.5 ml. (10 drops) of 0.01 *N* iodine solution (KI = 0.014 *M*) dropwise, with shaking. After 0.1 ml. (2 drops) has been added, the color of the solution is observed. At this point, the presence of small amounts of amylose in amylopectin will be evidenced by a blue color. The remainder of the 0.5 ml. of iodine is then slowly added and the color again observed. Further addition of iodine is unnecessary and in some cases may vitiate the color test by superimposing the color of free iodine on the starch-iodine color. All observations of color are made by transmitted daylight or a daylight fluorescent lamp and colors compared to the standard color chart.

SUMMARY

Starch-iodine color tests, to evidence small differences between starches or starch fractions and to be comparable among different laboratories, must be performed under uniform conditions in the absence of a large excess of iodine. A procedure is outlined for the rapid determination of starch-iodine colors. It is especially satisfactory for rapid estimation of the purity of amylopectin when contaminated with less than 6 to 8% amylose.

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A Versatile Arc-Spark Stand

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AN arc-spark stand combining the adjustments of the pin-type stand and the versatility of the Petrey stand (*1*) has been developed which is capable of supporting heavy weights and does not place any strain on the optical bar or the spectrograph. The size, shape, and vertical adjustments of the stand simplify nondestructive spectrographic analyses on relatively massive parts. In many laboratories this stand will broaden the scope of adaptability of the spectrograph, as either the pin technique (using fixtures) or the flat technique (*2*, *3*) may be used.

DESCRIPTION

A steel plate, 30 cm. (12 inches) square and 0.5 cm. ($^3/_{16}$ inch) thick, is used as the sample-supporting table. In the center of the edge of the sample plate towards the spectrograph slit a notch is cut, 2.5 cm. (1 inch) wide and 2.5 cm. (1 inch) deep, similar to the cut-out portion of the Petrey stand. The sample lying on the sample plate can easily be excited from the bottom, using the sample as one electrode and a graphite rod as the other (*2*, *3*). The notch must be large enough so that the excitation will be between the sample and the graphite rod and not between the sample plate and graphite rod. Notches of various size may be placed on the edges of the sample plate and the correct size found by trial. In this case, extra plate-holding screw holes are made in the top of the plate, in order that the plate may be turned to bring the proper notch toward the spectrograph slit.

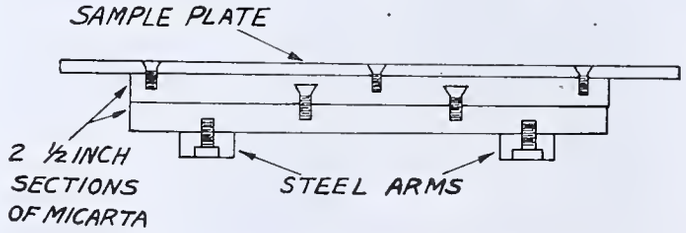

Figure 1. Method of Insulating Sample Plate

The sample plate is insulated from the remainder of the stand by two 1.8-cm. (0.5-inch) thick sections of micarta (Figure 1). All screws which fasten the micarta to the sample plate, etc., are offset from each other at least 2.5 cm. (1 inch). By this means at least 2.5 cm. (1 inch) of air or micarta insulate the sample plate from the remainder of the stand. The micarta is fastened to two

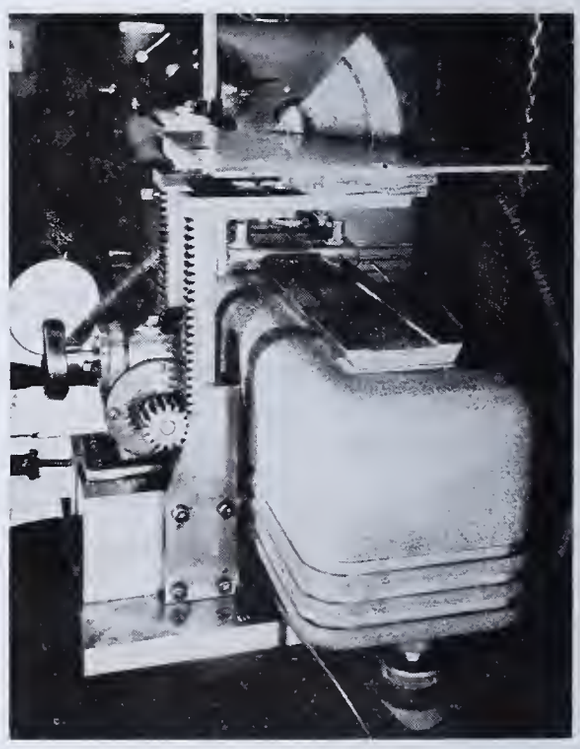

Figure 2. Arc-Spark Stand

solid pieces of steel which lead to one side of the optical bar, where they are attached to two bars of steel with teeth on the outer side which act as a rack for a rack and pinion motion.

A commercial-type speed reducer (15 to 1) with a reducer arm extending from each side was purchased and placed on a heavy steel platform about 10 cm. (4 inches) above the base of the stand (Figure 2). A pinion was attached to each reducer arm in such a manner as to contact the rack part of the uprights. On the motor extension part of the speed reducer a micarta knob was attached, to control the vertical motion of the sample plate.

The base, 28.5 cm. (11.25 inches) square and 2.5 cm. (1 inch) thick, rests on the table which supports the spectrograph (Bausch & Lomb large Littrow). Thus, the entire weight of the stand and sample is supported with no strain on the optical bar or the spectrograph.

All parts of the stand are of heavy, sturdy steel, to ensure rigidity. Although the stand is heavy (35 to 40 kg.), it is not cumbersome or bulky. It has supported over 50 kg. (100 pounds) without bending or twisting. With weights of this size, however, a thumb-type setscrew is placed on the side to contact one of the movable uprights. With smaller samples (5 to 10 kg.) this setscrew is not necessary, as the sample plate will remain in the position in which it is placed by the rack and pinion movement.

This stand is so constructed that the center of the notch in the center of the edge of the sample plate towards the spectrograph slit is exactly on the optical axis when the stand is placed firmly against the optical bar (Figure 2). In some instances, where the optical bar is of different design (Hilger, etc.), two slots in the base of the stand are advisable. Bolts can then be placed through these slots and through the supporting table. The stand can then be adjusted until it is properly aligned and the bolts tightened to give the same effect as at the author's laboratory, where the weight and position of the stand against the optical bar are used to hold the stand in proper alignment. Once the stand is in position, it is no longer necessary to move or adjust it, except with the rack and pinion motion.

The base of the stand is grounded to eliminate slight shocks due to induction from the sample plate, although the stand is usually adjusted by a gage before the sample is excited and it is not necessary to touch the stand during excitation.

Small fixtures to hold pins, etc., can easily be made to clip or screw onto the sample plate. Thus, the stand is used for many and varied types of samples, as either an upper or lower electrode holder as desired.

One lead from the source is attached to the sample plate by a countersunk screw, and the other lead to the portion of the regular B. & L. arc-spark stand which is used. The source leads are connected through a switch to convert from arc to spark as necessary. The stand is used at all times (with or without fixtures as necessary) as one pole of the source used.

The stand described has been in use over a year and can be recommended for various sizes, shapes, and weights of samples, either arc or spark. The direct current arc has been used for over an hour (arcing solutions with a fixture attached to the stand) with less than 1-minute interruptions every 5 minutes, without undue heating of the sample plate or stand.

ACKNOWLEDGMENTS

The author wishes to express his sincere gratitude to the Drafting and Tool Departments. He is particularly obliged to H. P. Conrad, senior chemist, for his active help.

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Apparatus for Vacuum Distillation of Volatile High-Melting Solids

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THE vacuum distillation of volatile high-melting solids necessitates laboratory apparatus of special design. In ordinary apparatus, the distilling solid soon clogs the narrow passage between the distilling vessel and the receiving vessel, particularly in the inaccessible places underneath stoppers and in the interior of the receiving vessel.

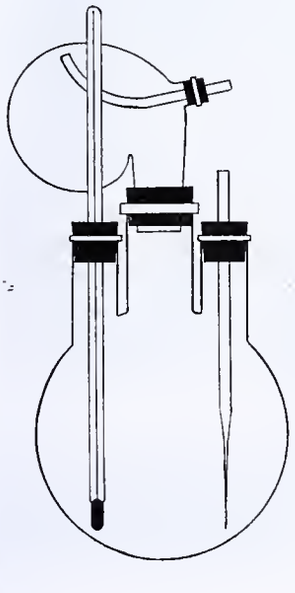
Two methods are used to overcome this difficulty. The first consists in heating the narrow parts to a temperature above the solidifying point of the distilling solid. Haehn (*3*) accomplished this by a hot oil jacket, Steinkopf (*8*) by an electrically heated wire-coil. Hauschild (*4*) used a specially designed still head which could be flamed directly. The second method consists in using specially designed flasks in which inaccessible narrow passages between the still and the receiver are eliminated. The most important apparatus of this type is the sword flask designed by Anschuetz (*6*), or its variations by Baer (*1*), Muencke (*6*), and others (*5*, *7*). Another has been described by Bolstad and Dunbar (*2*).

However, none of this equipment is readily available or available in sizes large enough to be practical for the handling of fairly large quantities of chemicals. A simple method was devised for this purpose, utilizing standard laboratory glassware (Pyrex).

The apparatus consists of a large three-necked flask (5 liters), and a retort (0.5 or 1 liter), the neck of which has been cut off conveniently. The shortened neck of the retort is inserted into the center neck of the flask (see figure). The other two necks of the flask hold a thermometer and a capillary tube. The vacuum connection is made through an upward-curved glass tube inserted into the tubulature of the retort. Heating is best carried out by inserting the flask deep into a suitable bath. Before charging, the flask should be tested for its ability to withstand a vacuum, taking adequate precautions. (Specially hand-picked flasks are available from Corning Glass Works. Caution must be used in all cases.)

The vacuum attachment clogged only when too much air was allowed to enter the apparatus through the capillary tube, and drops or particles of the distilland were carried up beyond the receiver. It was also found helpful to suspend, near the end of a distillation, a large test tube inside the center neck of the flask. This was done by means of a hook of noncorroding wire which was inserted through the neck of the retort and anchored to the sharp edge inside. By suspending the test tube within the center neck of the flask, the path of the distilling vapors is greatly shortened. Otherwise it would be necessary to overheat the contents of the flask to force the distillate up to the receiver.

An apparatus like the one described is available in large sizes, is comparatively inexpensive, and has operated satisfactorily in distilling fairly large amounts of material with only few interruptions.



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Apparatus for Trapping Ammonia in the Kjeldahl Method for Nitrogen

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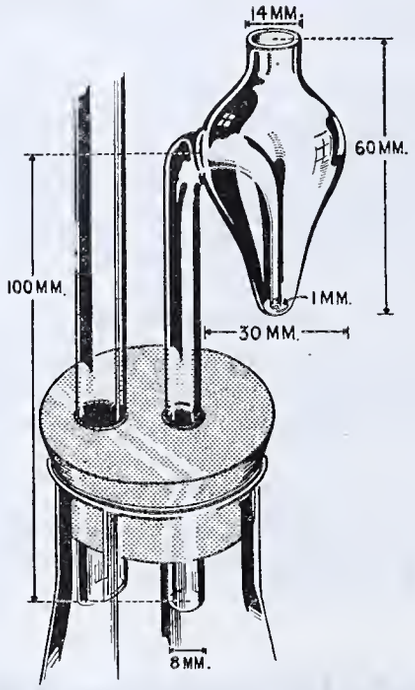
THE apparatus described below has been used satisfactorily in this laboratory for 20 years, to seal the receiver used in catching the ammonia distillate in the determination of nitrogen.



Used in analytical laboratories where nitrogen or protein is determined as a regular routine, the trap automatically catches the ammonia in the distillation step, eliminating the necessity of dipping the delivery tube into the standard acid. It offers several advantages: It does not require a delivery tube that must be removed from the solution before turning off the heat; a smaller quantity of solution may be used in the receiving flask; the action of the trap is automatic; its dependability has been proved by the laboratory's record in various collaborative check samples and it can be easily made by anyone experienced in working glass.

The block-tin delivery tube from the condenser and the tube from the trap extend through a No. 10 rubber stopper into a 500 ml. wide-necked Erlenmeyer flask used as the receiver. In operation about 2.5 cm. (1 inch) of water are put into the trap; more water is undesirable because of the danger of siphoning it out of the trap before completion of the distillation.

The apparatus will trap any ammonia vapors that are driven through the condenser by retaining them in solution. When the distillation is complete and the heat is turned off, the lower pressure inside the system will cause the solution in the trap to siphon into the receiving flask. The concentration of ammonia in the trap is very low. Experience has shown that it is not necessary to rinse the trap with water at the end of the distillation.

Using the trap and C.P. ammonium sulfate as a standard, recoveries have been found quantitative with amounts of nitrogen as high as 140 mg. per determination. The acid used is standardized according to the procedure of the Association of Official Agricultural Chemists (*1*).

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Aqueous Solutions of Alcohols as Confining Liquids for Gas Analysis

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THE use of solutions of alcohols, particularly glycerol, as confining liquids for gas analysis has been mentioned in numerous papers since it was first reported by Burgess and Wheeler (*1*). Using a solution of equal parts of glycerol and water previously saturated with coal gas, they reported that "the gases do not dissolve in such a mixture to any appreciable extent, and its use is more convenient than that of mercury". The advantage of the low freezing point of such a solution has been mentioned.

The authors have investigated the solubility of carbon dioxide in aqueous solutions of alcohols which might be satisfactory as confining liquids and compared these solubility data with those obtained for solutions of inorganic salts previously recommended. Carbon dioxide was selected because it is the most soluble gas commonly encountered in gas analysis. A survey of the literature (*4*) showed that carbon dioxide was sufficiently soluble in aqueous solutions of monohydric alcohols to preclude them from further consideration.

EXPERIMENTAL

The apparatus and technique are those employed previously (*2*, *3*). The organic compounds were of reagent quality or were vacuum-distilled. Dissolved gases were removed from the water and the compounds (except glycerol and dihydroxy diethyl ether) by refluxing for 20 minutes or by vacuum distillation. The absorption bulb and gas buret were maintained at 25° ± 0.1° C. The carbon dioxide (99.8% purity) was saturated with water vapor from the solution by passage through a spiral-type bubbler maintained at 25° C. Values of the vapor pressure of the water in the solution were taken from the literature or calculated from

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Table I. Solubility of Carbon Dioxide in Aqueous Alcohol Solutions

(Temperature 25° C., partial pressure of CO_2 760 mm.)

Compound Used	Concentration	Solution Used	Gas Absorbed	Solubility of CO_2	
	Wt. %	*Ml.*	*Ml.*	*Ml.*[a]	*α*[b]
None	0.0	24.99	20.57	0.823	0.7
Glycerol	40.0	50.00	29.62	0.592	0.5
Glycerol	50.0	50.00	25.55	0.512	0.4
Glycerol	50.0	50.00	23.10	0.462	0.4
Sulfuric acid	5.0				
Ethylene glycol	60.0	49.99	31.36	0.627	0.5
Ethylene glycol	40.0	50.00	32.68	0.654	0.5
Ethylene glycol	20.0	50.00	36.31	0.726	0.6
Dioxane[c]	60.0	50.00	76.13	1.523	1.3
β-β′-Dihydroxy ethyl ether[c]	60.0	24.99	15.80	0.633	0.5
Tetrahydrofurfuryl alcohol[c]	75.0	50.00	55.66	1.113	1.0

[a] Ml. of CO_2 at 25° C. and 760 mm. dissolved per ml. of solution.
[b] Ml. of CO_2 calculated to 0° C. and 760 mm. dissolved per ml. of solution
[c] This solution reacted with mercury.

Raoult's law. The total pressure maintained in the apparatus was 760 mm. of carbon dioxide plus the vapor pressure of the solution at 25° C.

The solubility data are given as milliliters of carbon dioxide at 760 mm. and 25° C. dissolved in 1 ml. of solution. From this is calculated the Bunsen coefficient, α, which is the milliliters of carbon dioxide, calculated to 760 mm. and 0° C., dissolved in 1 ml. of solution (*4*). Although the precision of the results is 0.002, it can be considered that the accuracy of α is 0.005. The data are given in Table I.

DISCUSSION

The Bunsen coefficient for an acidified 50% glycerol solution (0.423) is almost twice that found for the 20% sodium sulfate plus 5% sulfuric acid solution (0.247) previously recommended (*2*, *3*). Although sodium sulfate decahydrate will crystallize from the acidified sodium sulfate solution below 16° C., this solution will continue to dissolve less gas than the acidified glycerol solution. Only at temperatures below the cryohydric point would the acidified glycerol solution have advantages. The cryohydric temperature of saturated aqueous sodium sulfate solution is −1.10° and of sodium chloride is −21.1° C. An acidified aqueous solution of sodium chloride is recommended for use at temperatures to −20° C. (−4° F.).

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Third paper in series; for Nos. 1 and 2 see (*2*, *3*).

Electronic Timer

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RECENT work in the physical chemistry laboratory of this bureau involved the use of a solenoid-operated, enclosed glass pump. The electronic timing device described by Bechtold (*1*) appeared particularly well suited for controlling this solenoid because of its simplicity, ease of construction, and readily controllable pulse rate. However, this timer proved unsatisfactory in operation since, under the conditions which obtained, the gas-filled triode (885) had an average service life of only about 12 hours. No current-limiting element was included in the triode plate circuit. Thus, the charging surge supplied to capacitor C_2 (Bechtold's designation) was, upon breakdown of the triode, in excess of the rated peak current for the tube. This overload caused the tube to fail prematurely. Insertion of resistance to limit the peak current to the specified maximum (300 milliamperes) resulted in feeble actuation of the relay. Satisfactory operation required the use of a more sensitive relay, or of a tube having a higher peak current rating.

In the improved circuit given in Figure 1 the type 885 tube has been replaced by a type 2050 gas-filled tetrode and a current-limiting resistor has been inserted. This resistor, R_6, has a value of about 170 ohms and limits the charging surge of C_2 to less than the rated maximum (1 ampere) of the 2050 tube. Resistor R_5 has been added to limit grid current and rheostat R_7 to reduce arcing of the relay contacts on an inductive load, such as a direct current solenoid.

The modified circuit has proved highly satisfactory as evidenced by over 200 hours of operation, including continuous periods of as long as 60 hours, with no evidence of tube failure. Its readily adjustable pulse rate, obtained by manipulation of potentiometer R_1 and the adjustability of the range of rates depending upon the values of R_1, R_2, and C_1, make it applicable to a wide variety of timing operations.

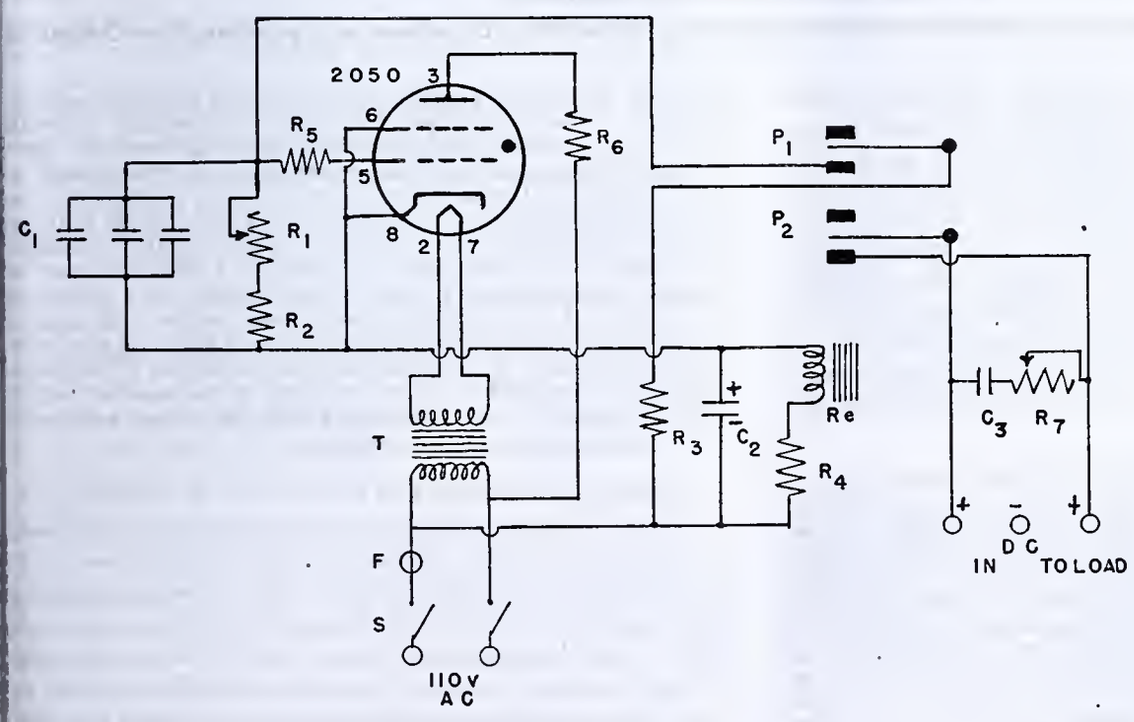

Figure 1. Circuit for Electronic Timer

C_1, 3 paper condensers, 400 wv, 2 μfd. each; C_2, 250 wv. electrolytic condenser, 8 μfd.; C_3, paper condenser, 450 wv., 1 μfd.; R_1, 1-megohm potentiometer, No. 6 taper; R_2, 50,000 Ω, 1-watt carbon; R_3, R_4, 10,000 Ω, 1-watt carbon; R_5, 1-megohm, 1-watt carbon; R_6, 150 Ω 5-watt wire-wound; R_7, 100 Ω, 2-ampere rheostat; T, 6.3-volt 3-ampere filament transformer; Re, double-pole, double-throw relay, 2000 Ω coil; F, 2.0-ampere fuse; S, double-pole, single-throw toggle switch; 2050, RCA type 2050 gas-filled tetrode; P_1, P_2 relay contact points

ACKNOWLEDGMENT

Suggestions made by P. J. Franklin in connection with the design of this circuit are gratefully acknowledged.

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Gage for Preparation of Laboratory Solutions

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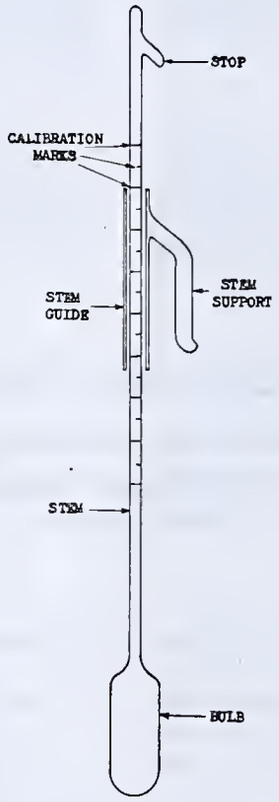
THE use of a 1- or 2-liter volumetric flask for making large quantities of standard solutions up to 19 liters (5 gallons) is somewhat tedious and time-consuming. While clear-glass bottles can be calibrated by placing a mark at the desired point, this method cannot be used for opaque bottles and has the additional disadvantage that the bottle must be level.

A bottle gage embodying the float principle is presented in the diagram and furnishes a simple, rapid, and accurate method for making up solutions in large bottles.



The gage consists of a hollow bulb on the end of a stem, together with a guide and support for the stem. The bulb must be of such size and shape that it will pass through the mouths and necks of all bottles to be used and at the same time float in distilled water. The stem support must have enough clearance between it and the guide tube to accommodate all bottles.

The gage for 2-, 3-, and 5-gallon bottles used in this laboratory has the following dimensions:

Bulb. Length, 5.5 cm.; greatest width, 2.3 cm.; volume displacement, 14.0 cc.

Stem. Length, 32.0 cm.; diameter, 0.5 cm.

Stem Guide. Length, 9.0 cm.; inside diameter, 0.6 cm.

Support. Clearance between support and guide, 1.5 cm.

The gage is calibrated by measuring the desired quantity of distilled water into a bottle of suitable size, placing the gage in position, and making a mark on the stem even with the top of the guide tube. This process is repeated for other quantities and bottles as desired. However, the calibrations for bottles of any given size may be used for all bottles of the same size provided that they are of approximately the same diameter and height.

In general, to make up a chemical solution the calculated amount of chemical or chemicals is put in the empty bottle, the gage is placed in position, and distilled water is added to the proper mark. The solution is mixed well and then standardized, adjustments being made when necessary.

Since the gage operates near the center of the solution with respect to the circumference of the bottle, its accuracy is not affected if the bottle is not exactly level. The slight difference between the specific gravity of the unmixed solution and that of distilled water does not materially affect its accuracy.

A suitable holder for the gage is made by placing a small piece of cotton in a 500-cc graduate.

ACKNOWLEDGMENT

The author is indebted to J. H. Jordan for making the gage as well as for suggestions concerning details.

Di- and Triethylene Glycols as Manostat Fluids

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DEVICES for automatically controlling reduced pressure have numerous laboratory applications. In carrying out exacting laboratory vacuum distillations involving use of high-efficiency fractionating columns of 100 theoretical plates or more and operating at high reflux ratios (100 to 1), it is essential that the column pressure be controlled closely. Even minor fluctuations in pressure will cause serious disturbance of the equilibrium between liquid and vapor carefully established along the entire length of the column. Since such precise fractionations may extend over periods of a month or even more, the pressure control should not only be very precise but also provide trouble-free operations.

In the laboratory of the Naval Stores Research Division various terpene hydrocarbons are commonly distilled at a pressure of 20 mm. of mercury. A Palkin-type oil gage (*4*) which can be easily read to 0.5 mm. of oil (and, therefore, to approximately 0.03 mm. of mercury) is used, and column pressure is maintained at 293.0 mm. of oil (corresponding to 20.0 mm. of mercury) to approximately this precision. Five columns are operated from a single "column line" manifold controlled by a triethylene glycol manostat, and individual distillations have been continued for as long as 5 weeks. The same manostat has been in use for over a year without replacement and without requiring any attention other than resetting.

A dual system of pressure control involving an automatically controlled gas leak from the higher pressure column line to the lower pressure vacuum pump line is used. The low pressure pump line is maintained at approximately 10 mm. of mercury by means of a vacuum pump controlled by an ordinary mercury manostat. The pump operates only at infrequent intervals. Fine control of pressure in the column line is achieved by use of the triethylene glycol Hershberg-Huntress–type (*2*) manostat, an electronic relay (Cenco-Gilson electronic relay obtainable from the Central Scientific Co., Chicago, Ill., Catalog No. 99,780), and a solenoid-operated breather valve (Model K-20-1 obtainable from the General Controls Co., Glendale, Calif.). Surges are minimized by adequate ballast and by insertion of a 15-cm. (6-inch) length of capillary tubing in the breather line. In practice there is no perceptible fluctuation of the level of the oil gage when the solenoid opens or closes, although slight motion of the manostat fluid is perceptible. The frequency of the audible clicking of the solenoid valve as it opens and closes provides a good guide to the presence of leaks in the system.

According to Hershberg and Huntress (*2*), a desirable manostat fluid is a "liquid which will combine electrical conductivity, low density, low vapor pressure, and reasonable viscosity with the property of wetting the manostat wall". These investigators recommended sulfuric acid of specific gravity 1.71 as a manostat fluid to control a thermionic relay. Acid of this concentration, although superior to ordinary concentrated sulfuric acid, is not entirely satisfactory, as it is corrosive and hygroscopic and easily becomes fouled with grease from the stopcocks. In addition, some electrolysis occurs and the electrodes become pitted.

The Cenco-Gilson electronic relay, which is reported to operate on as little as 0.5 microampere, permits the use of a practically

Table I. Comparative Properties of Manostat Fluids

Substance	Specific Gravity at 20° C.	Viscosity at 20° C. *Centipoises*	Boiling Point at 760 Mm. ° C.	Vapor Pressure at 25° C. *Mm.*
Mercury	13.55 (*3*)	1.547 (*3*)	357.9 (*3*)	0.0018 (*3*)
80% H_2SO_4	1.7272 (*3*)	20.3 (*3*) (at 25° C.)	202 (*3*)	0.124 (*3*)
75% H_2SO_4	1.6692 (*3*)	14.0 (*3*) (at 25° C.)	182 (*3*)	0.408 (*3*)
Diethylene glycol	1.1184 (*3*)	35.7 (*1*)	244.8 (*1*)	0.1 (*1*) (0.1 at 45° C.)
Triethylene glycol	1.1254 (*1*)	47.8 (*1*)	287.3 (*1*)	0.01 (*1*) (0.1 at 80° C.)

nonconducting manostat fluid, therefore materially enlarging the field from which a suitable fluid may be selected. Both diethylene glycol and triethylene glycol, which are readily available commercially, have been found superior to sulfuric acid for use as manostat fluids. Both these glycols have sufficient electrical conductivity to operate the Cenco-Gilson electronic relay, but when they are used with the thermionic relay suggested by Hershberg and Huntress, a drop of concentrated sulfuric acid must be added to provide sufficient conductivity.

The significant properties of the substances discussed above as possible manostat fluids are compared in Table I.

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A Rapid-Filling Capillary Polarimeter Tube

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IN THE course of an extended study of the optical rotations of several scarce materials, the limited quantities of sample available necessitated the use of capillary polarimeter tubes. The ordinary polarimeter tubes which are generally used in sugar analysis (*1*) and are readily available have an inside diameter of about 9 mm. With a tube of this bore, it would have been necessary either to reduce the length to a few millimeters or to use extremely dilute solutions. Since the rotation is a function of the length of tube and concentration of the solution, neither alternative was feasible. Compared with the usual 9-mm. diameter tube, the 2-mm. bore tube employed by the authors requires only 5% of the solution volume for an equal tube length.

Many investigators have employed capillary tubes such as the Fischer microtube (*2*) the Naumann tube (*3*) or various modifications of them. The filling of small-bore tubes of these types presents definite difficulties. It is impossible to pour the liquid into the vertically held tube while one of the cover glasses is fastened to the lower end. If any air bubbles are trapped in the course of the filling, it is generally necessary to empty the entire sample before attempting to refill free of air bubbles.

This difficulty is usually overcome by introducing the solution from a long thin dropper which will extend to the bottom of the capillary (*2*). Withdrawing the dropper as the liquid enters the tube permits filling with a fair degree of success. However, the fragility of the long dropper and the ever-present danger of losing the sample if the dropper is broken are disadvantages.

The "halo" caused by light scattered from the inside walls of small-bore tubes makes it difficult to obtain a well-defined balance of the photometric field. Naumann (*3*) overcomes this difficulty by employing black glass capillary tubes with etched inside walls, but admits that proper cleaning of the etched tube is a problem in itself.

The authors have reduced this halo effect to a minimum by limiting the field of the polarimeter. When a diaphragm is mounted in the threaded end cap of the tube nearest the analyzer, with its hole diameter so selected that it subtends a smaller angle to the observer than does the end of the capillary bore nearest the polarizer, the halo is eliminated at the expense of a small reduction in the diameter of the photometric field.

In order to obtain satisfactory tubes for their investigation, the authors have devised a new type of capillary tube by the simple expedient of introducing into an ordinary straight-bore, 200-mm. polarimeter tube a piece of capillary whose outside diameter makes a rather snug sliding fit (about 0.01-mm. clearance) in the inside of the regular tube. These sections of capillary are optically polished on both ends. Their total length is made approximately 0.5 mm. shorter than that of the regular tube, so that the original tube length still remains effective when rotation measurements are made.

While using these assembled capillary tubes it became apparent that they can be filled easily, rapidly, and safely by putting the necessary amount of solution into the large tube and then slowly lowering the capillary into it. The lowering of the capillary displaces the solution, which rises into the bore completely free of bubbles. The solution also rises in the annular space between the tube and the capillary, but this does not interfere, nor does it use much of the solution. For a tube of the dimensions stated, the annular volume is about 0.015 ml.

If the solution does not come all the way to the top, when the capillary is lowered in the tube, a small amount may easily be added from a short dropper.

In many laboratories it is necessary to have a large number of polarimeter tubes of different bore size to accommodate the various quantities of solutions to be measured on the polarimeter. By means of a series of different-size capillaries which may be readily constructed, a single observation tube may be quickly converted into a capillary tube of the proper bore. Water-jacketed tubes without tubulatures may be similarly converted to capillary tubes, bearing in mind, of course, that the time required for attaining temperature equilibrium will be greatly increased because of the increased thickness of tube wall.

SUMMARY

Polarimetric measurements on small amounts of the sample require the use of capillary tubes. As a result of optical rotation studies on several small samples, a rapid-filling capillary polarimeter tube has been developed. For small volumes of solutions, this tube gives the maximum tube length which is concomitant with a sufficiently large field to permit photometric matching.

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Electrolyte Solution Heating Element for Steam Microbath

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A SIMPLE, rapid, and efficient heating device particularly adapted for use as an electrically operated steam bath can be constructed by immersing two wire electrodes in a solution of an electrolyte of proper concentration. The apparatus (Figure 1) has an automatic cutoff feature and requires no external transformer or resistance control.

The apparatus is a vessel of about 200-ml. capacity covered by a metal water bath adapter through which the electrodes pass. The electrodes are two pieces of No. 12 copper wire bared for a length of 4 cm. and insulated from the adapter by Lucite bushings. The wires fit snugly into holes drilled through the Lucite. One of the bushings is cut to permit the electrode to be partially raised out of the solution, thereby altering the effective size of the electrode. Two parallel grooves, each the thickness of the metal plate and 1 cm. apart, are cut in a 2.5-cm. length of 0.94-cm. (0.375-inch) diameter Lucite rod. The section below the uppermost groove is then filed to have two parallel flat faces, the material down to the depth of the groove being removed. The opening in the metal plate is made by drilling a hole and filing until the lower end of this bushing will pass through. Then by raising the bushing to the level of the lower groove and rotating it 90° the area of the electrode is reduced, thereby decreasing rate of heating.

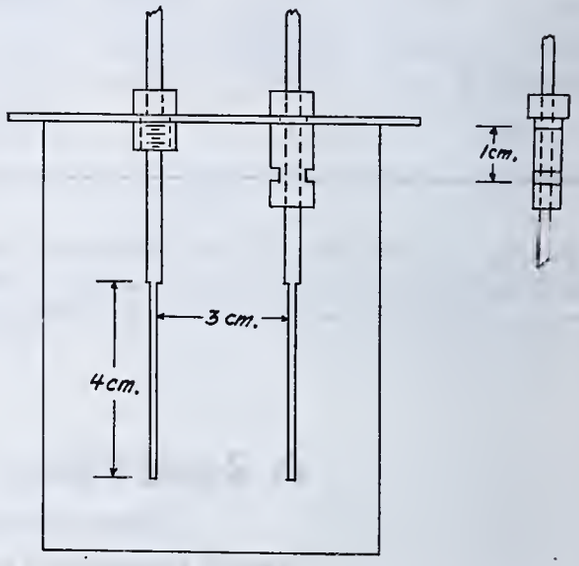

Figure 1. Details of Apparatus, Including Side View of Special Bushing

A 1% copper sulfate solution proved satisfactory as the electrolyte. Some idea of the time to reach the boiling point may be useful: 100 ml. of solution produced steam in less than 1.5 minutes after the unit was plugged into the commercial power lines.

The automatic cutoff is obtained by suspending the electrodes so that they do not touch the bottom of the vessel. Then as the level of the solution drops below the lower edge of the electrodes the circuit is broken. As the electrolyte remains in the residue, the addition of water will recharge the apparatus.

Semimicro Ion-Exchange Column

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IN RECENT months the writer has had an opportunity to study the ion-exchange adsorption of a large number of organic compounds. In order to obtain basic facts as to adsorption capacity of exchangers and recovery data it was found expedient to use small quantities of exchange materials.

The ion-exchange columns recommended by investigators in the water-conditioning field (*1–4*) were found to be oversized (200- to 1000-ml. exchanger beds) and cumbersome to operate. A need was felt for a column which would hold from 5 to 20 ml. of exchanger and could easily be assembled, backwashed, or connected in series with another column.

Such an apparatus was constructed by using a 9-mm. standard Kimble condenser tube measuring 42.5 cm. long when the beveled end had been cut off. A plug of glass wool was inserted at the bottom and a three-way stopcock was attached by means of a rubber stopper.

A complete assembly of two columns is shown in Figure 1. The solution to be run enters C^1 by means of a funnel and constant-head device. After flowing through the exchange bed in the first column it passes to the second by means of a tube leading from B^1 to C^2. Samples from the first column may be taken at any time through A^1 by turning the stopcock.

The effluent passes out of the second column through A^2 and its flow rate may be adjusted by means of the stopcock.

The columns may be backwashed by attaching a tube from a raised water bottle to B^2 as illustrated or to A^1 for the first column and providing an overflow tube.

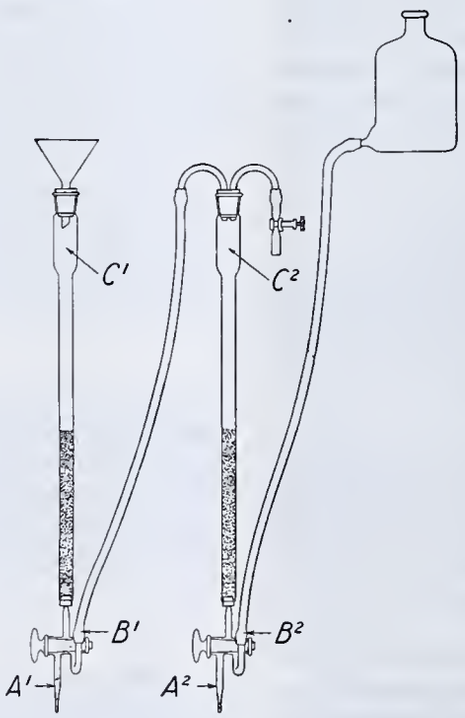

Figure 1

Even at moderately rapid backwash rates the exchangers remained suspended in the wide part of the tube at C^1C^2 and showed no tendency to be spilled over. No trouble was caused by entrapped air, as is usual in larger columns. Flow rates could be adjusted with fair accuracy over a wide range. Where necessary, the tube may be calibrated to study bed volume changes.

This apparatus has been found useful in studies on the ion-exchange adsorption and recovery of alkaloids, amino acids, and other organic compounds.

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Tabulated Diffraction Data for Tetragonal Isomorphs

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COMPOUND analysis of crystalline solids by isomorphism depends for its utility on the availability of systematically arranged representative diffraction patterns of the various known crystal structures. The tabulation of the cubic structures (*3*) has been found useful and the effectiveness of the method has prompted the compilation of the tetragonal structures. The procedure for comparing diffraction patterns of isomorphous substances has been described adequately (*4*), hence only the tabulated diffraction data for tetragonal isomorphs are presented in this paper.

Figures 1, 2, 3, and 4 depict representative diffraction patterns of 40 tetragonal structures designated as in the "Strukturbericht" (*2*, *5–9*). The patterns are arranged in sets and within each set the simplest structure with the highest symmetry (*11*) is listed first. The averaged relative intensities, based largely on the Dow file of standards, refer to Debye-Scherrer-Hull patterns taken with MoKα radiation. For each structure only reflections compatible with the respective space group are shown. The intensities of superposed lines were resolved by calculating the appropriate structure factors. To round out the available powder data, some fifty substances were synthesized and their diffraction patterns carefully indexed.

GENERAL PROCEDURE FOR IDENTIFYING A NONCATALOGED PATTERN

(1) Plot the log d values and corresponding relative intensities of the unidentified pattern on a narrow strip of paper; (2) verify that the pattern is noncubic (*3*); (3) find an isomorphic prototype among the representative diffraction patterns for the anisotropic crystal structures; (4) compute the lattice constants and check the appropriate classification tables (for the tetragonal system check Tables III and IV); (5) confirm the identification of the unknown phase by a qualitative spectroscopic analysis or by spot tests.

The following example illustrates the procedure:

Table I. X-Ray Powder Diffraction Data

d, kX	$\frac{I}{I_1}$	$\{hkl\}$
7.25	0.06	001
3.75	1.00 (100)	101
3.60	0.15	002
3.10	1.00 (100)	110
(2.98)	(0.01)	
2.78	0.63	102
2.35	0.75 (75)	112
2.19	0.63	200
2.11	0.25	201, 103
1.90	0.40	211, 113
1.72	0.25	212
1.68	0.20	104
1.62	0.02	203
(1.59)	(0.01)	
1.55	0.15	220, 114
1.52	0.15	221, 213
1.43	0.08	222, 301
1.39	0.10	204, 310
1.355	0.02	311, 302

Filtered MoKα was used to obtain the powder diffraction data. d = nterplanar spacing. $\frac{I}{I_1}$ = relative intensity. The lattice constants for the unknown phase are $a = 4.38\ kX$, $c = 7.23\ kX$; $\frac{c}{a} = 1.65$.

Table II. Statistical Data on Tabulated Tetragonal Substances

Dominant structures	HO_4, C4, C11, OB20, ...
N_r = number of examples	36, 33, 18, 17, ...
$\frac{N_r}{\Sigma_r N_r}$	0.11, 0.10, 0.06, 0.05, ...
Configurations favoring tetragonal symmetry	Tetrahedral, octahedral, square; linear; $4n$-cyclic
Prevalence of primitive lattices	53%
Prevalence of body-centered lattices	47%
$\bar{n}_{hko}$ = average number of observed prism reflections per powder pattern	4.4; $1 \leq n_{hko} \leq 9$
$\bar{\nu}_{hko}$ = probability of observing $\{hko\}$	$(\bar{\nu}_{110}, \bar{\nu}_{200}, \bar{\nu}_{220}) = (0.65, 0.93, 0.90)$
$\frac{\bar{I}_{hko}}{\bar{I}_1}$ = average relative intensity of $\{hko\}$	$\left(\frac{\bar{I}_{110}}{\bar{I}_1}, \frac{\bar{I}_{200}}{\bar{I}_1}, \frac{\bar{I}_{220}}{\bar{I}_1}\right) = (0.38, 0.51, 0.17)$
$_n\{hkl\}$ = probability that $\{hkl\}$ is the nth strongest powder reflection of a tetragonal structure	$\bar{\nu}_1\{101, 112, 110, 211, 200\} = (0.30, 0.22, 0.13, 0.13, 0.08)$ $\bar{\nu}_2\{101, 200, 211, 110, 112\} = (0.15, 0.13, 0.10, 0.08, 0.08)$ $\bar{\nu}_3\{110, 211, 101, 200, 112\} = (0.10, 0.10, 0.08, 0.08, 0.05)$
$\frac{\bar{c}}{\bar{a}}$ = average axial ratio	1.51; $0.31 \leq \frac{c}{a} \leq 6.7$

Columns 1 and 2 of Table I give the powder diffraction data of a phase encountered in a magnesium flux sample. After plotting log d and the corresponding relative intensities on a strip of paper, one checks first the index scale for the cubic system (*3*) and finds that the substance is noncubic. Proceeding to the tetragonal system and comparing systematically the unknown pattern with those of Figures 1, 2, 3, and 4, one makes the following observations: The first indication of a fit with the data is noted for **C11** with $\frac{c}{a} = 1.64$ ($a \approx 4.4\ kX$, $c \approx 7.2\ kX$). However, the lines 7.25, 2.78, 1.72, 1.68, 1.62 kX are not accounted for by this structure and the intensity agreement of the indexed lines is not satisfactory. Structure **C38** permits indexing all the lines for $\frac{c}{a} = 1.65$ ($a = 4.38\ kX$, $c = 7.23\ kX$) but gives poor agreement with the relative intensities. Checking Table III under **C38**, one fails to find a substance agreeing with the computed lattice constants. A similar situation is encountered for structure $\mathbf{D3_1}$ with $\frac{c}{a} = 2.32$ ($a = 6.20\ kX$, $c = 14.5\ kX$) and for structure $\mathbf{DO_{22}}$ with $\frac{c}{a} = 1.65$ ($a = 8.75\ kX$, $c = 14.5\ kX$). Structure $\mathbf{EO_1}$, however, accounts for all the lines and matches the relative intensities well. Looking under $\mathbf{EO_1}$ for $\frac{c}{a} = 1.65$ ($a = 4.38\ kX$, $c = 7.23\ kX$), one identifies the unknown as BaFCl. Spectroscopic analysis confirms Ba as a major constituent. The faint lines 2.98 and 1.59 kX belong to an unidentified material present in low concentration. (In completing the comparison of the identified pattern with the remaining tetragonal structures, one finds only partial matching with structures $\mathbf{HO_4}$, $\mathbf{HO_7}$, and $\mathbf{HO_8}$.)

An alternative method of identifying the unknown phase is based on the general tabulation of tetragonal substances in Table IV. By matching the unknown pattern against the standard structures it is noted that the pattern can be indexed tetragonal with $\frac{c}{a} = 1.65$ ($a = 4.38$ kX, $c = 7.23$ kX). Looking in Table IV for these lattice constants one finds BaFCl listed. As in the first method a qualitative spectroscopic analysis is required to confirm the phase identification. In using Table IV it is advisable to look not only under $\frac{c}{a}$ but also under $\left(\frac{2^r p}{q}\right)\frac{c}{a}$ where $p, q = 1,2,3,\ldots,n$ and $r = 0, \pm\frac{1}{2}$. In practice one usually considers only the factors $2, \sqrt{2}, \frac{1}{\sqrt{2}}, \frac{1}{2}$.

The ease with which a tetragonal pattern can be recognized is a determining factor in the speed of the above methods. Reference to Table II suggests how one may expedite the indexing of tetragonal powder patterns. For approximately 85% of the tetragonal substances it is possible to pick out the {200} and {220} reflections by translating the log d strip of the unknown pattern along the {hko} scale at the top of Figure 1. The data of Table I can be used to illustrate the procedure. After plotting log d one fixes the first line, $d_1 = 7.25$ kX, on {200} of the {hko} scale and notes if any of the remaining d's match {220}. As no match is found one proceeds systematically to the succeeding lines and observes that $d_4 = 3.10$ kX and $d_8 = 2.19$ kX match—i.e., $d_4:d_8 = 1.415 \approx \sqrt{2} = d_{\{200\}}:d_{\{220\}} = d_{\{110\}}:d_{\{200\}}$. Checking the possibility that $d_4:d_8 = d_{\{110\}}:d_{\{200\}}$, one sees immediately that $d_4:d_8:d_{15} = 3.10:2.19:1.55 = d_{\{110\}}:d_{\{200\}}:d_{\{220\}} = 2:\sqrt{2}:1$. Three prism reflections are thus identified and the unknown pattern is readily indexed by following the {110} lines down Figure 1 until a match is found for $\frac{c}{a} = 1.65$. The phase identification is then carried out in the manner already discussed.

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Table III. Tetragonal Substances Tabulated by Types

Table III lists 329 tetragonal substances. For each isomorphous type the members are arranged in the ascending order of their axial ratios. If two members have the same value for c/a the one with the smaller lattice constants is listed first. (To avoid repetition, the various literature values have been averaged.) For specific references prior to 1939 see "Strukturbericht", Vols. I to VII: for later references see *Chem. Abst.*, 33 to 39 (1939–1945).

$\frac{c}{a}$	a, kX	c, kX	Substance
		A5	
0.5456	5.819	3.175	β-Sn
		A6	
0.936	3.774	3.533	γ-Mn
0.952	3.76	3.58	95Mn.5Cu
0.962	3.767	3.624	89Mn.11Cu
0.981	3.764	3.693	79Mn.21Cu
0.998	3.752	3.744	66Mn.34Cu
1.077	4.585	4.937	In
		B10	
1.22	3.55	4.33	LiOH
1.26	3.98	5.01	PbO (red)
1.27	3.80	4.81	SnO
		B17	
1.74	3.03	5.26	PdO
1.76	3.03	5.32	PtO
1.76	3.47	6.10	PtS
		B25 and OB25	
0.667	6.011	4.009	NH_4SH
0.707	6.18	4.37	γ-NH_4I
0.709	5.70	4.04	γ-NH_4Br($\sim$173° K.)
0.711	7.78	5.53	$N(CH_3)_4Cl$
0.712	8.44	6.01	$N(CH_3)_4MnO_4$
0.713	7.76	5.53	$N(CH_3)_4Br$
0.722	8.28	5.98	$N(CH_3)_4ClO_4$
0.724	7.94	5.75	$N(CH_3)_4I$
0.729	6.34	4.62	PH_4I
		B34	
1.03	6.37	6.58	(Pd, Pt, Ni)S
1.04	6.35	6.60	PdS
		B37	
0.873	8.02	7.00	TlSe
		NaBi	
1.35	3.24	4.38	MgIn
1.39	3.46	4.80	NaBi
		C4	
0.570	(4.86)	(2.77)	WO_2
0.574	(4.86)	(2.79)	MoO_2
0.621	4.77	2.96	CbO_2
0.633	4.61	2.92	$RhVO_4$
0.634	4.54	2.88	VO_2
0.642	4.69	3.01	$RhCbO_4$
0.644	4.58	2.95	TiO_2
0.645	4.68	3.02	$RhTaO_4$
0.649	4.41	2.86	CrO_2
0.649	4.64	3.01	$CrCbO_4$
0.650	4.63	3.01	$CrTaO_4$
0.651	4.39	2.86	GeO_2
0.652	4.40	2.87	MnO_2
0.652	4.62	3.01	$FeSbO_4$
0.652	4.67	3.04	$FeTaO_4$
0.652	4.68	3.05	$FeCbO_4$
0.656	4.51	2.96	$AlSbO_4$
0.659	4.64	3.06	MgF_2
0.660	4.59	3.03	$GaSbO_4$
0.660	4.71	3.11	NiF_2
0.664	4.58	3.04	$CrSbO_4$
0.665	4.72	3.14	ZnF_2
0.672	4.72	3.17	SnO_2
0.674	4.60	3.10	$RhSbO_4$
0.678	4.87	3.30	MnF_2
0.679	4.70	3.19	CoF_2
0.683	4.95	3.38	PbO_2
0.686	4.93	3.38	PdF_2
0.690	4.51	3.11	RuO_2
0.696	4.83	3.36	FeF_2
0.699	4.49	3.14	IrO_2
0.707	4.51	3.19	OsO_2
0.787	(4.79)	(3.77)	TeO_2
		C5	
2.51	3.75	9.43	TiO_2
		C11	
1.15	6.27	7.22	CsO_2
1.17	5.99	7.02	RbO_2
1.18	5.70	6.73	KO_2
1.20	...	...	UC_2
1.28	(4.14)	(5.28)	ThC_2
1.61	4.39	7.05	BaC_2
1.63	4.11	6.68	SrC_2
1.65	3.82	6.30	NdC_2
1.65	3.87	6.37	CaC_2
1.66	3.85	6.38	PrC_2
1.67	3.54	5.91	CaO_2
1.67	3.75	6.28	SmC_2
1.67	3.87	6.48	CeC_2
1.67	3.92	6.55	LaC_2

(*Continued on next page*)

Table III (*Continued*)

$\frac{c}{a}$	a, kX	c, kX	Substance
			BaO_2
1.79	3.78	6.77	BaO_2
1.85	3.55	6.55	SrO_2
1.97	(4.28)	(8.42)	KHC_2
2.10	(3.89)	(8.17)	$NaHC_2$
			C13
2.83	4.36	12.36	HgI_2
			C16
0.806	6.052	4.878	Al_2Cu
0.814	6.52	5.31	Sn_2Fe
0.818	6.647	5.434	Sn_2Mn
0.832	5.099	4.240	Fe_2B
0.841	5.006	4.212	Co_2B
0.846	5.899	4.991	Ge_2Fe
0.851	4.980	4.236	Ni_2B
0.852	6.835	5.821	Pb_2Pd
0.857	6.348	5.441	Sn_2Co
0.880	6.651	5.853	Pb_2Rh
			C20
2.45	3.21	7.88	WSi_2
2.46	3.20	7.86	$MoSi_2$
			C30
1.40	4.96	6.92	SiO_2
1.41	5.00	7.06	$AlPO_4$
			C38
1.53	3.992	6.091	Cu_2Sb
1.61	4.08	6.56	Mn_2Sb
1.65	3.627	5.973	Fe_2As
1.67	3.76	6.27	Mn_2As
1.75	3.613	6.333	Cr_2As
			C47
0.605	8.35	5.05	SeO_2
			C48
2.88	2.998	8.630	Cr_2Al
			ZnP_2
3.68	5.07	18.65	ZnP_2
3.73	5.28	19.70	CdP_2
			DO_{22}
1.46	5.548	8.093	$TiGa_3$
1.55	5.605	8.712	$ZrGa_3$
1.56	5.334	8.305	VAl_3
1.57	5.422	8.536	$TaAl_3$
1.58	5.425	8.579	$TiAl_3$
1.58	5.427	8.584	$CbAl_3$
			DO_{23}
4.32	4.003	17.29	$ZrAl_3$
			$D1_3$
2.46	4.53	11.14	Al_4Ba
2.48	4.45	11.04	Al_4Sr
2.54	4.35	11.07	Al_4Ca
			$D3_1$
2.36	4.92	11.62	Hg_2I_2
2.39	4.65	11.10	Hg_2Br_2
2.44	4.46	10.89	Hg_2Cl_2
2.98	3.66	10.9	Hg_2F_2
			$D5_9$
1.40	8.75	12.28	Cd_3P_2
1.41	8.10	11.45	Zn_3P_2
1.41	8.32	11.76	Zn_3As_2
1.41	8.95	12.65	Cd_3As_2
			Fe_3P
0.490	9.09	4.45	Fe_3P
0.491	9.01	4.42	$(Fe, Ni, Co)_3P$
0.492	8.92	4.39	Ni_3P
0.499	9.13	4.56	Cr_3P
0.502	9.16	4.60	Mn_3P
			EO_1
1.65	4.38	7.22	BaFCl
1.68	4.10	6.88	SrFCl
1.71	4.65	7.93	BaFI
1.76	3.89	6.83	CaFCl
1.76	4.09	7.21	PbFCl
1.82	4.18	7.59	PbFBr
1.89	3.89	7.37	BiOCl
2.07	3.92	8.11	BiOBr
2.28	4.01	9.14	BiOI
			$E1_1$
1.82	5.66	10.30	$AgFeS_2$
1.97	5.26	10.37	$CuFeS_2$
			$E1_4$
0.550	10.81	5.94	$(PnCl_3)_4$
			$E2_3$
1.89	4.19	7.94	NH_4HgCl_3
			$E2_6$
0.502	13.30–13.52	6.69–6.78	$KMg(H_2O)_6(Cl, Br)_3$
			$(E3_1)$
1.00	6.34	6.34	Ag_2HgI_4
1.01	6.08	6.14	Cu_2HgI_4
			$(E6_1)$
0.906	(6.41)	(5.81)	$Sr(OH)_2.8H_2O$
			$(E6_2)$
0.880	(6.32)	(5.56)	$SrO_2.8H_2O$
			$FeTa_2O_6$
1.94	4.70	9.10	$NiTa_2O_6$
1.94	4.71	9.12	$Fe(Cb, Ta)_2O_6$
1.94	4.73	9.16	$CoTa_2O_6$
1.95	4.70	9.18	$MgTa_2O_6$
1.95	4.71	9.18	$FeTa_2O_6$
			$F1_1$
0.921	9.69	8.92	$Hg(CN)_2$
			$F5_2$
1.16	6.07	7.03	KNCO
1.16	6.09	7.06	KN_3
1.17	6.36	7.41	RbN_3
1.20	5.67	6.81	KFHF
			$F5_4$
0.593	6.31	3.74	NH_4ClO_3
			GO_9
0.871	5.74	5.00	NH_4NO_3-II (357–398° K.)
			Phosgenite
1.086	8.13	8.83	$Pb_2Cl_2CO_3$
1.086	8.34	9.06	$Pb_2Br_2CO_3$
			HO_3
0.867	7.13	6.18	YVO_4
0.874	7.25	6.34	$CaCrO_4$
0.888	6.87	6.10	YPO_4
0.901	6.58	5.93	$ZrSiO_4$
0.910	6.89	6.27	$YAsO_4$
1.46	(7.74)	(11.31)	$Y(Cb, Ta)O_4$
1.46	(7.76)	(11.32)	$YCbO_4$
1.47	(7.75)	(11.41)	$YTaO_4$
			HO_4
2.15	5.94	12.80	NH_4IO_4
2.17	5.15	11.17	$CdMoO_4$
2.17	5.24	11.38	$CaWO_4$
2.17	5.35	11.63	$NaLa(WO_4)_2$
2.18	5.32	11.59	$NaCe(WO_4)_2$
2.18	5.34	11.63	$LiLa(WO_4)_2$
2.19	5.23	11.44	$CaMoO_4$
2.19	5.27	11.55	$NaBi(MoO_4)_2$
2.19	5.36	11.72	$NaReO_4$
2.20	5.23	11.50	$LiBi(MoO_4)_2$
2.20	5.31	11.67	$LiLa(MoO_4)_2$
2.20	5.33	11.70	$NaLa(MoO_4)_2$
2.20	5.40	11.90	$SrWO_4$
2.20	5.75	12.63	KIO_4
2.20	5.87	12.94	$RbIO_4$
2.20	5.87	12.94	NH_4ReO_4
2.21	5.44	12.01	$PbWO_4$
2.21	5.44	12.03	$KLa(WO_4)_2$
2.22	5.38	11.92	$KBi(MoO_4)_2$
2.22	5.39	11.94	$KCe(WO_4)_2$
2.22	5.62	12.50	$KReO_4$
2.23	5.35	11.92	$AgReO_4$
2.23	5.37	11.96	$SrMoO_4$
2.23	5.41	12.08	$PbMoO_4$
2.23	5.42	12.11	$KLa(MoO_4)_2$
2.24	5.32	11.93	$NaIO_4$
2.24	5.37	12.01	$AgIO_4$
2.26	5.62	12.70	$BaWO_4$
2.27	5.80	13.17	$RbReO_4$
2.29	5.56	12.76	$BaMoO_4$
2.30	5.08	11.69	$BiAsO_4$
2.31	5.76	13.33	β-$TlReO_4$ (400° K.)
2.32	5.65	13.08	$KOsO_3N$
2.36	5.46	12.89	$KCrO_3F$
2.52	5.61	14.13	$CsSO_3F$
2.53	5.72	14.50	$CsCrO_3F$

(*Continued on page 87*)

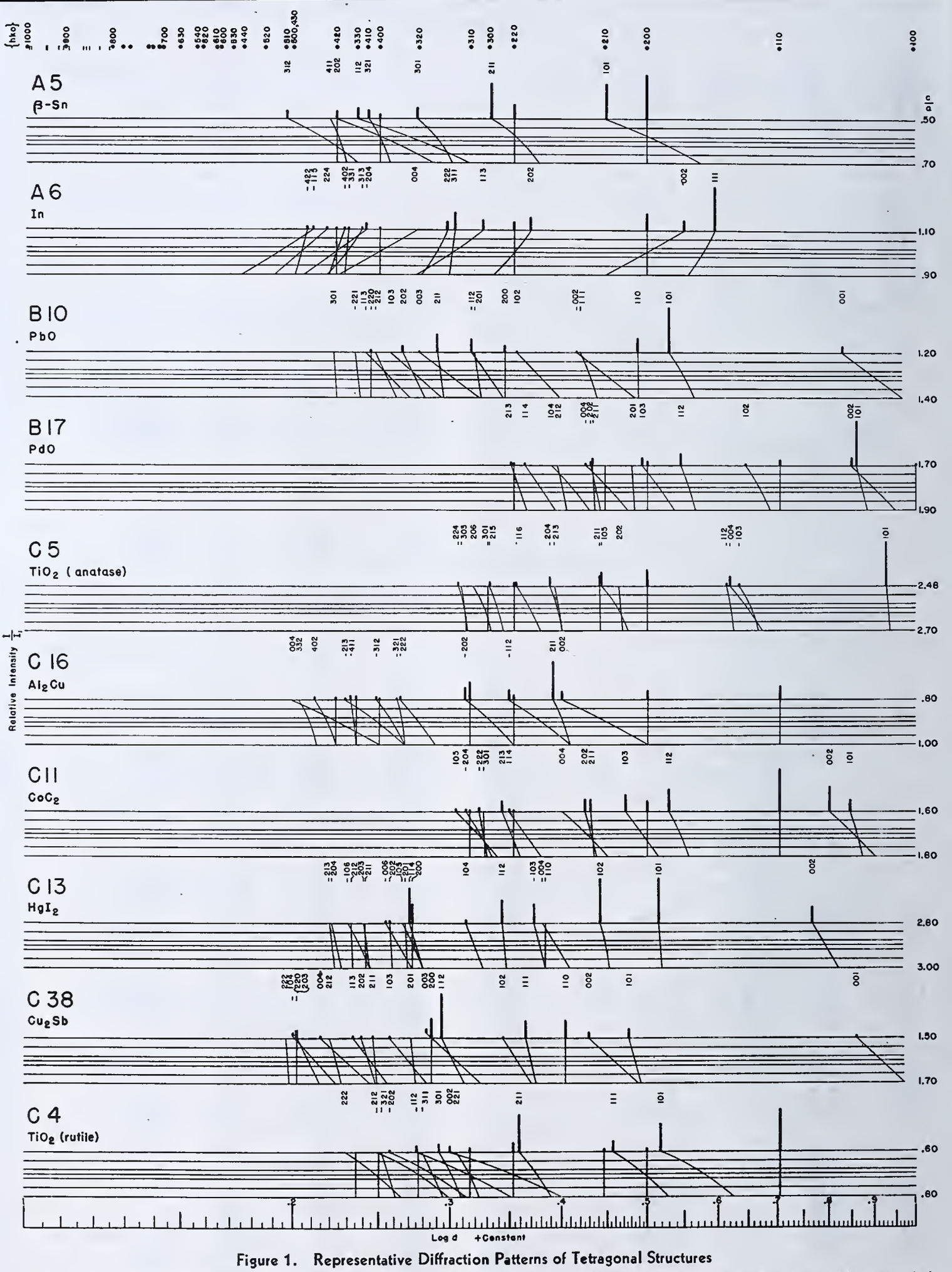

Figure 1. Representative Diffraction Patterns of Tetragonal Structures

The log d_{hko} values of the various structures (Figures 1, 2, 3, and 4) are matched according to the $\{hko\}$ reflections. (To conserve printing space some of the structures had to be translated to the left—e.g., *B10, C13, C38.*) The relative intensities of the powder pattern pertaining to a particular structure represent the arithmetically averaged relative intensities (for MoKα radiation) of representative members of the isomorphous group. The change of d_{hkl} with c/a is expressed graphically (*1, 10*) and covers the c/a spread encountered within the particular isomorphous group

Table III (*Continued*)

$\frac{c}{a}$	a, kX	c, kX	Substance
		HO_7	
1.524	4.459	6.796	$BAsO_4$
1.533	4.332	6.640	BPO_4
		HO_8	
1.73	3.55	6.14	$KAlF_4$
1.73	3.62	6.26	$RbAlF_4$
1.76	3.61	6.37	$TlAlF_4$
1.77	3.59	6.35	NH_4AlF_4
		($H1_1$)	
1.48	5.85	8.68	$CuFe_2O_4$
1.58	6.20	9.82	$CaIn_2O_4$
1.59	5.74	9.15	$ZnMn_2O_4$
1.61	6.12	9.87	$CdIn_2O_4$
1.64	5.75	9.42	$MnMn_2O_4$
		$PbPb_2O_4$	
0.684	8.22	5.62	$NiAs_2O_4$
0.687	8.592	5.905	$FeSb_2O_4$
0.689	8.685	5.980	$MnSb_2O_4$
0.696	8.49	5.91	$CoSb_2O_4$
0.697	8.491	5.920	$ZnSb_2O_4$
0.699	8.445	5.907	$MgSb_2O_4$
0.708	8.35	5.91	$NiSb_2O_4$
0.722	8.72	6.30	$SnPb_2O_4$
0.742	8.85	6.57	$PbPb_2O_4$
		$H1_6$	
0.582	7.04	4.10	K_2PdCl_4
0.591	6.99	4.13	K_2PtCl_4
0.591	7.21	4.26	$(NH_4)_2PdCl_4$
		$H2_2$	
0.936	7.61	7.12	KH_2AsO_4
0.938	7.43	6.97	KH_2PO_4
1.003	7.52	7.54	$NH_4H_2PO_4$
1.005	7.70	7.74	$NH_4H_2AsO_4$
		$H2_6$	
1.97	5.46	10.73	Cu_2FeSnS_4
		$H4_1$	
1.024	7.81	8.00	$Rb_2CuCl_4.2H_2O$
1.05	7.58	7.96	$(NH_4)_2CuCl_4.2H_2O$
1.05	7.9	8.3	$(NH_4)_2CuBr_4.2H_2O$
1.06	7.45	7.88	$K_2CuCl_4.2H_2O$
1.09	7.50	8.16	$(NH_4)_2FeCl_4.2H_2O$
		$H4_9$	
0.403	10.40	4.19	$Pt(NH_3)_4Cl_2.H_2O$
0.423	10.21	4.31	$Pd(NH_3)_4Cl_2.H_2O$
		$H4_{17}$	
0.753	8.43	6.35	$Ag_2SO_4.4NH_3$
		$H5_9$	
2.95	6.99	20.63	$Ca(UO_2)_2(PO_4)_2.10^1/_2H_2O$
		$H5_{10}$	
1.21	6.98	8.42	$Ca(UO_2)_2(PO_4)_2.6^1/_2H_2O$
		$La_2(MoO_4)_3$	
2.18	5.32	11.60	$Pr_2(MoO_4)_3$
2.19	5.29	11.58	$Nd_2(MoO_4)_3$
2.21	5.33	11.78	$Ce_2(MoO_4)_3$
2.21	5.35	11.84	$La_2(MoO_4)_3$
2.23	5.22	11.62	$Sm_2(MoO_4)_3$
		$NaBaPO_4$	
0.847	9.76	8.27	$NaBaPO_4$
0.848	9.84	8.34	$KBaPO_4$
0.857	9.25	7.93	$NaSrPO_4$
0.859	9.50	8.16	$KSrPO_4$
		$J1_5$	
1.25	6.99	8.75	$K_2Os_2O_2Cl_4$
		$J1_9$	
1.50	6.97	10.43	$AgCo(NH_3)_2(NO_2)_4$
		$J1_{11}$	
0.974	8.12	7.91	$AgSb(OH)_3$
0.984	8.01	7.88	$NaSb(OH)_3$
		$J3_1$	
1.14	15.84	18.01	$K_3TlCl_6.2H_2O$
1.15	16.95	19.45	$Rb_3TlBr_6.8/7H_2O$
		$K3_1$	
1.58	9.18	14.47	Cs_3CoCl_5
1.61	8.7	14.0	Rb_3CoCl_5

$\frac{c}{a}$	a, kX	c, kX	Substance
		$K3_4$	
1.71	8.39	14.34	$NH_4Pb_2Br_5$
1.72	8.41	14.5	$RbPb_2Br_5$
1.73	8.14	14.1	KPb_2Br_5
		$K7_5$	
1.48	7.01	10.36	$Na_5Al_3F_{14}$
		$K7_6$	
1.45	7.49	10.87	$Cs_2AuAuCl_6$
1.49	7.38	11.01	$Cs_2AgAuCl_6$
		K_3CrO_8	
1.13	6.70	7.60	K_3CrO_8
1.13	7.37	8.34	Cs_3TaO_8
1.14	7.05	8.05	Rb_3TaO_8
1.16	6.78	7.86	K_3CbO_8
1.16	6.78	7.88	K_3TaO_8
		$LiBi_3O_4Cl_2$	
3.13	3.840	12.03	$LiBi_3O_4Cl_2$
3.13	3.877	12.13	$NaBi_3O_4Cl_2$
3.20	3.925	12.55	$NaBi_3O_4Br_2$
3.20	3.943	12.62	$Cd_2Bi_2O_4Br_2$
3.22	3.876	12.47	$LiBi_3O_4Br_2$
3.34	3.970	13.24	$Cd_2Bi_2O_4I_2$
3.34	3.990	13.31	$NaBi_3O_4I_2$
3.35	3.941	13.19	$LiBi_3O_4I_2$
		L10	
0.825	3.89	3.21	NiZn
0.935	3.98	3.72	AuCu
0.966	3.85	3.72	FePd
0.967	3.66	3.54	NiMn
		$S2_3$	
0.757	15.63	11.83	$Ca_{10}Mg_2Al_4Si_9O_{34}(OH)_4$
		$S5_2$	
1.76	9.00	15.84	$KCa_4Si_8O_{20}F.8H_2O$
		$S5_3$	
0.637	7.83	4.99	$Ca_2ZnSi_2O_7$
0.651	7.76	5.05	$(Ca, Na)_2(Mg, Al)(Al, Si)_2O_7$
0.655	7.75	5.08	$Ca_2Al_2SiO_7$
0.659	(7.47)	(4.92)	$(Ca, Na)_2Be(Al, Si)_2(O, F)_7$
0.675	(7.38)	(4.98)	$(Ca, Na)_2BeSi_2(O, OH, F)_7$
		$S6_4$	
0.624	12.27	7.66	$Ca_4Al_6Si_6O_{24}(SO_4, CO_3)$
0.627	12.09	7.58	$Na_4Al_3Si_9O_{24}Cl$
		OB11	
0.632	9.18	5.80	$N(CH_3)_4ICl_2$
		OB20	
1.72	5.09	8.76	CH_3NH_3Br
1.75	5.11	8.96	CH_3NH_3I
2.95	5.18	15.3	$C_4H_9NH_3I$
2.96	5.02	14.85	$C_4H_9NH_3Cl$
3.03	5.02	15.23	$C_4H_9NH_3Br$
3.33	5.01	16.7	$C_5H_{11}NH_3Cl$
3.35	5.16	17.3	$C_5H_{11}NH_3I$
3.40	5.00	17.0	$C_5H_{11}NH_3Br$
3.78	5.16	19.50	$C_6H_{13}NH_3I$
3.93	4.98	19.55	$C_6H_{13}NH_3Cl$
4.02	4.93	19.8	$C_6H_{13}NH_3Br$
4.15	5.18	21.5	$C_7H_{15}NH_3I$
4.25	4.96	21.1	$C_7H_{15}NH_3Cl$
4.58	5.18	23.7	$C_8H_{17}NH_3I$
5.42	5.18	28.1	$C_{10}H_{21}NH_3I$
5.88	5.18	30.46	$C_{11}H_{23}NH_3I$
6.03	5.18	31.24	$C_{12}H_{25}NH_3I$
		OB21	
1.20	4.28	5.13	CH_3NH_3Cl
1.51	4.85	7.33	$C_3H_7NH_3I$
1.58	4.48	7.10	$C_3H_7NH_3Cl$
1.61	4.57	7.36	$C_3H_7NH_3Br$
		OB25 and OHO_5	
0.711	7.78	5.53	$N(CH_3)_4Cl$
0.712	8.44	6.01	$N(CH_3)_4MnO_4$
0.713	7.76	5.53	$N(CH_3)_4Br$
0.722	8.28	5.98	$N(CH_3)_4ClO_4$
0.724	7.94	5.75	$N(CH_3)_4I$
		$Tl(CH_3)_2I$	
2.81	4.78	13.43	$Tl(CH_3)_2I$
3.08	4.47	13.78	$Tl(CH_3)_2Br$
3.27	4.29	14.02	$Tl(CH_3)_2Cl$

(*Continued on page 89*)

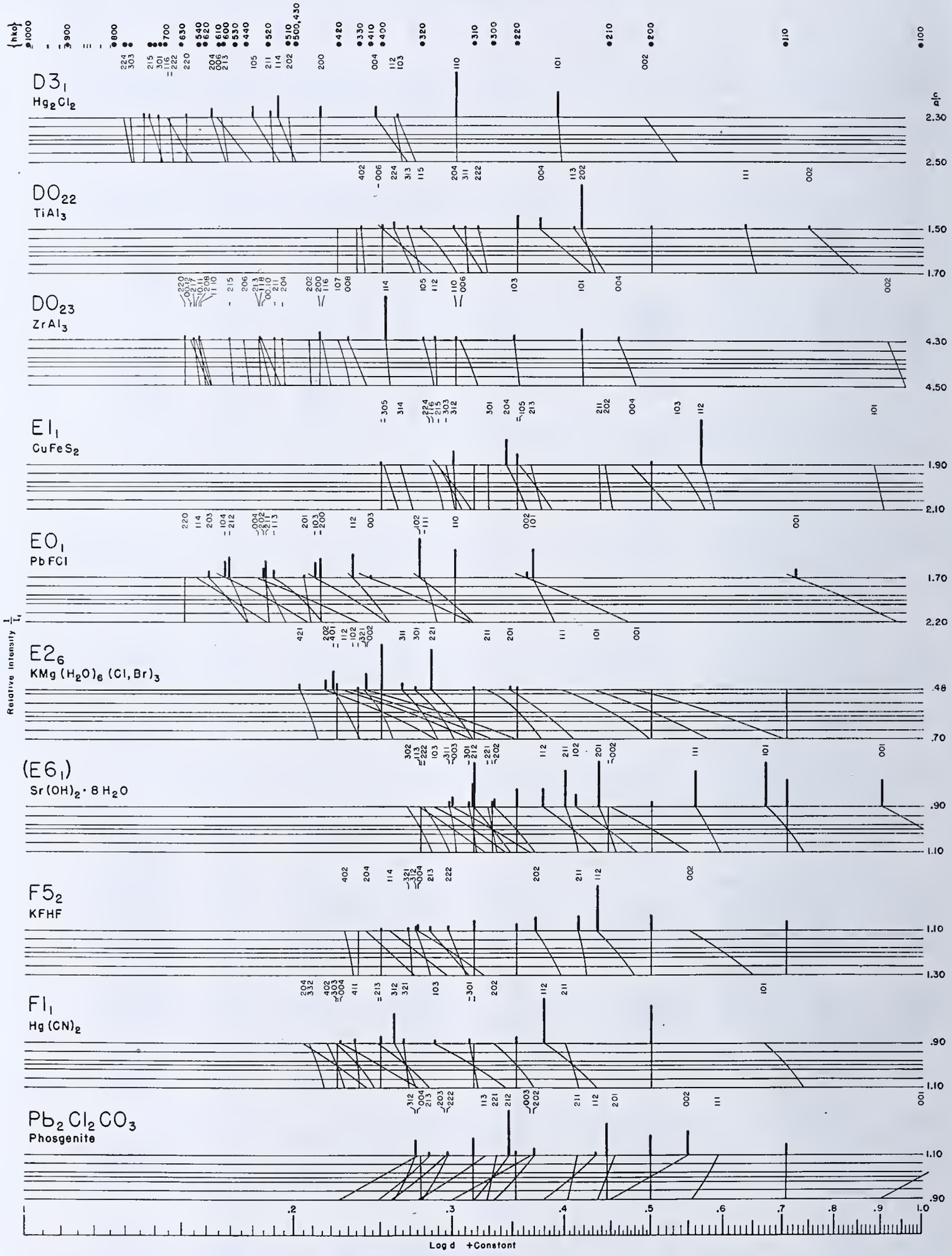

Figure 2. Representative Diffraction Patterns of Tetragonal Structures

Table III (*Continued*)

$\frac{c}{a}$	a, kX	c, kX	Substance
		$OG1_2$	
0.451	16.1	7.26	$[(CH_3)_3As, PdCl_2]_2$
0.451	16.6	7.48	$[(CH_3)_3As, PdBr_2]_2$
		$OJ1_1$	
1.42	7.88	11.19	$[N(CH_3)_4]_2SiF_6$
		$[(CH_3)_2SiO]_8$	
0.613	13.95	8.55	$[(CH_3)_2SiO]_8$
		$O1_2$	
0.396	10.37	4.11	$[CH_3CHO]_4$
		$O2_6$	
0.427	12.59	5.37	$Ag(CH_3.CS.NH_2)_4Cl$
0.444	12.43	5.52	$Cu(CH_3.CS.NH_2)_4Cl$
		$O4_{12}$	
1.44	6.09	8.76	$C(CH_2OH)_4$
		$O5$	
0.457	12.1	5.53	$C(CH_2OCOCH_3)_4$
		$O6$	
0.533	12.15	6.48	$Pb(C_6H_5)_4$
0.544	11.90	6.47	$Sn(C_6H_5)_4$
0.591	11.60	6.85	$Ge(C_6H_5)_4$
0.612	11.44	7.00	$Si(C_6H_5)_4$
0.645	11.09	7.15	$C(C_6H_5)_4$
0.713	(9.38)	(6.69)	$C(CH_2ONO_2)_4$

Table IV. Tetragonal Substances Tabulated According to Axial Ratios

Table IV lists 447 tetragonal substances arranged in ascending order of their axial ratios. If two substances have the same value for c/a, the one with the smaller lattice constants is listed first. Column 4 also includes space group data where no definite structure has been established.

$\frac{c}{a}$	a, kX	c, kX	Type	Substance
0.311	12.2	3.79	...	$Na_{(0.2-0.4)}WO_3$
0.367	11.44	4.20	S_4^2-$I\bar{4}$	$Cd[Hg(CNS)_4]$
0.394	11.09	4.37	S_4^2-$I\bar{4}$	$Co[Hg(CNS)_4]$
0.396	10.37	4.11	$O1_2$	$[CH_3CHO]_4$
0.401	11.06	4.43	S_4^2-$I\bar{4}$	$Zn[Hg(CNS)_4]$
0.403	10.40	4.19	$H4_9$	$Pt(NH_3)_4Cl_2.H_2O$
0.417	10.12	4.22	...	Be-(W,Mo)
0.422	10.21	4.31	$H4_9$	$Pd(NH_3)_4Cl_2.H_2O$
0.427	12.59	5.37	$O2_6$	$Ag(CH_3.CS.NH_2)_4Cl$
0.429	14.6	6.26	...	$MgPt(CN)_4.7H_2O$
0.444	12.43	5.52	$O2_6$	$Cu(CH_3.CS.NH_2)_4Cl$
0.451	16.1	7.26	$OG1_2$	$[(CH_3)_3As, PdCl_2]_2$
0.451	16.6	7.48	$OG1_2$	$[(CH_3)_3As, PdBr_2]_2$
0.457	12.1	5.53	$O5$	$C(CH_2OCOCH_3)_4$
0.464	8.12	3.77	...	CS_2(~100° K.)
0.487	18.8	9.15	C_{4h}^6-$I4_1/a$	$C_6H_4[1,2]CH_3.SO_2NH_3$
0.490	9.09	4.45	Fe_2P	Fe_2P
0.491	9.01	4.42	Fe_2P	$(Fe,Ni,Co)_2P$
0.492	8.92	4.39	Fe_2P	Ni_2P
0.495	7.56	3.74	...	W_4O_{11}
0.499	9.13	4.56	Fe_2P	Cr_2P
0.502	9.16	4.60	Fe_2P	Mn_2P
0.502	13.30–13.52	6.69–6.78	$E2_6$	$KMg(H_2O)_6(Cl,Br)_3$
0.514	34.04	17.49	D_{4h}^{14}-$P4/mnm$	$NaK(Ca,Mg,Mn)$-$Al_4Si_5O_{18}.8H_2O$, ashcroftine
0.518	5.56	2.88	...	CdHg
0.533	12.15	6.48	$O6$	$Pb(C_6H_5)_4$
0.536	12.78	6.85	C_{4h}^6-$I4_1/a$	$CH_2OH(CHOH)_2$-CH_2OH
0.541	26.60	14.40	...	$(C_6H_4[1,2]O.$-$CH{=}NOH)_2Pt$
0.544	11.90	6.47	$O6$	$Sn(C_6H_5)_4$
0.546	5.819	3.175	$A5$	β-Sn
0.550	10.81	5.94	$E1_4$	$[PNCl_2]_4$
0.550	12.17	6.69	...	$AgClO_2$
0.552	7.82	4.32	...	$ZnHg(CNS)_4$
0.570	(4.86)	(2.77)	$C4$	WO_2
0.574	(4.86)	(2.79)	$C4$	MoO_2
0.582	7.04	4.10	$H1_5$	K_2PdCl_4
0.582	12.19	7.09	S_4^2-$I\bar{4}$	$(C_6H_5)_4AsI$
0.591	6.99	4.13	$H1_5$	K_2PtCl_4
0.591	7.21	4.26	$H1_5$	$(NH_4)_2PdCl_4$
0.591	11.60	6.85	$O6$	$Ge(C_6H_5)_4$
0.593	6.31	3.74	$F5_4$	NH_4ClO_3
0.603	9.22	5.56	...	$Na_2Co(CNS)_4.8H_2O$. julienite
0.605	8.35	5.05	$C47$	SeO_2
0.612	11.44	7.00	$O6$	$Si(C_6H_5)_4$
0.613	13.95	8.55	$[(CH_3)_2SiO]_5$	$[(CH_3)_2SiO]_8$
0.617	4.81	2.97	$C4$	CbO_2
0.622	(5.72)	3.56	C_{4h}^5-$I4/m$	Ni_4Mo
0.624	12.27	7.66	$S6_4$	$Ca_4Al_6Si_6O_{24}(SO_4,CO_3)$ meionite
0.627	12.09	7.58	$S6_4$	$Na_4Al_3Si_3O_{24}Cl$, marialite
0.632	9.18	5.80	$OB11$	$N(CH_3)_4ICl_2$
0.633	4.61	2.92	$C4$	$RhVO_4$
0.634	4.54	2.88	$C4$	VO_2
0.637	7.83	4.99	$S5_4$	$Ca_2ZnSi_2O_7$, hardystonite
0.642	4.69	3.01	$C4$	$RhCbO_4$
0.644	4.58	2.95	$C4$	TiO_2
0.645	4.68	3.02	$C4$	$RhTaO_4$
0.645	11.09	7.15	$O6$	$C(C_6H_5)_4$
0.649	4.41	2.86	$C4$	CrO_2
0.649	4.64	3.01	$C4$	$CrCbO_4$
0.650	4.63	3.01	$C4$	$CrTaO_4$
0.651	4.39	2.86	$C4$	GeO_2
0.651	4.67	3.04	$C4$	$FeTaO_4$
0.651	7.76	5.05	$S5_3$	$(Ca,Na)_2(Mg,Al)$-$(Al_2Si)_2O_7$ melilite
0.652	4.40	2.87	$C4$	MnO_2
0.652	4.62	3.01	$C4$	$FeSbO_4$
0.652	4.68	3.05	$C4$	$FeCbO_4$
0.655	7.75	5.08	$S5_3$	$Ca_2Al_2SiO_7$, gehlenite
0.656	4.51	2.96	$C4$	$AlSbO_4$
0.659	4.64	3.06	$C4$	MgF_2
0.659	(7.47)	(4.92)	$S5_3$	$(Ca,Na)_2Be(Al,Si)_2$-$(O,F)_7$, meliphanite
0.660	4.59	3.03	$C4$	$GaSbO_4$
0.660	4.71	3.11	$C4$	NiF_2
0.664	4.58	3.04	$C4$	$CrSbO_4$
0.665	4.72	3.14	$C4$	ZnF_2
0.667	6.011	4.009	$B25$	NH_4SH
0.672	4.72	3.17	$C4$	SnO_2
0.674	4.60	3.10	$C4$	$RhSbO_4$
0.675	(7.38)	(4.98)	$S5_3$	$(Ca,Na)_2BeSi_2$-$(O,OH,F)_7$, leucophanite
0.678	4.87	3.30	$C4$	MnF_2
0.679	4.70	3.19	$C4$	CoF_2
0.683	4.95	3.38	$C4$	PbO_2
0.684	8.22	5.62	$PbPb_2O_4$	$NiAs_2O_4$
0.684	12.32	8.43	...	$C(CH_2OC_6H_5)_4$
0.686	4.93	3.38	$C4$	PdF_2
0.687	8.592	5.905	$PbPb_2O_4$	$FeSb_2O_4$
0.689	8.685	5.980	$PbPb_2O_4$	$MnSb_2O_4$
0.690	4.51	3.11	$C4$	RuO_2
0.696	4.83	3.36	$C4$	FeF_2
0.696	8.49	5.91	$PbPb_2O_4$	$CoSb_2O_4$
0.697	8.491	5.920	$PbPb_2O_4$	$ZnSb_2O_4$
0.699	4.49	3.14	$C4$	IrO_2
0.699	8.445	5.907	$PbPb_2O_4$	$MgSb_2O_4$
0.707	4.51	3.19	$C4$	OsO_2
0.707	6.18	4.37	$B25$	γ-NH_4I
0.708	8.35	5.91	$PbPb_2O_4$	$NiSb_2O_4$
0.709	5.70	4.04	$B25$	γ-NH_4Br (~173° K.)
0.711	7.78	5.53	$OB25$	$N(CH_3)_4Cl$
0.712	8.44	6.01	$OB25$	$N(CH_3)_4MnO_4$
0.713	7.76	5.53	$OB25$	$N(CH_3)_4Br$
0.713	(9.38)	(6.69)	$O6$	$C(CH_2ONO_2)_4$
0.715	8.56	6.12	$A18$	Cl_2 (88° K.)
0.718	10.15	7.29	D_4^6-$P4_22_1$	$OsO_3C_4(CH_3)_8$
0.718	12.04	8.65	...	$Ca(OCl)_2.3H_2O$
0.722	8.28	5.98	$OB25$	$N(CH_3)_4ClO_4$
0.722	8.72	6.30	$PbPb_2O_4$	$SnPb_2O_4$
0.724	7.94	5.75	$OB25$	$N(CH_3)_4I$
0.729	6.34	4.62	$B25$	PH_4I
0.731	16.53	12.09	...	Cd_3Hg
0.739	10.76	7.95	...	$Na_2(TiFe)Si_4O_{11}$, narsarsukite
0.742	8.85	6.57	$PbPb_2O_4$	$PbPb_2O_4$
0.752	9.74	7.32	...	Cu_3Pd
0.753	8.43	6.35	$H4_{17}$	$Ag_2SO_4.4NH_3$
0.757	15.63	11.83	$S2_3$	$Ca_{10}Mg_2Al_4Si_9O_{34}(OH)_4$, vesuvianite

(*Continued on page 91*)

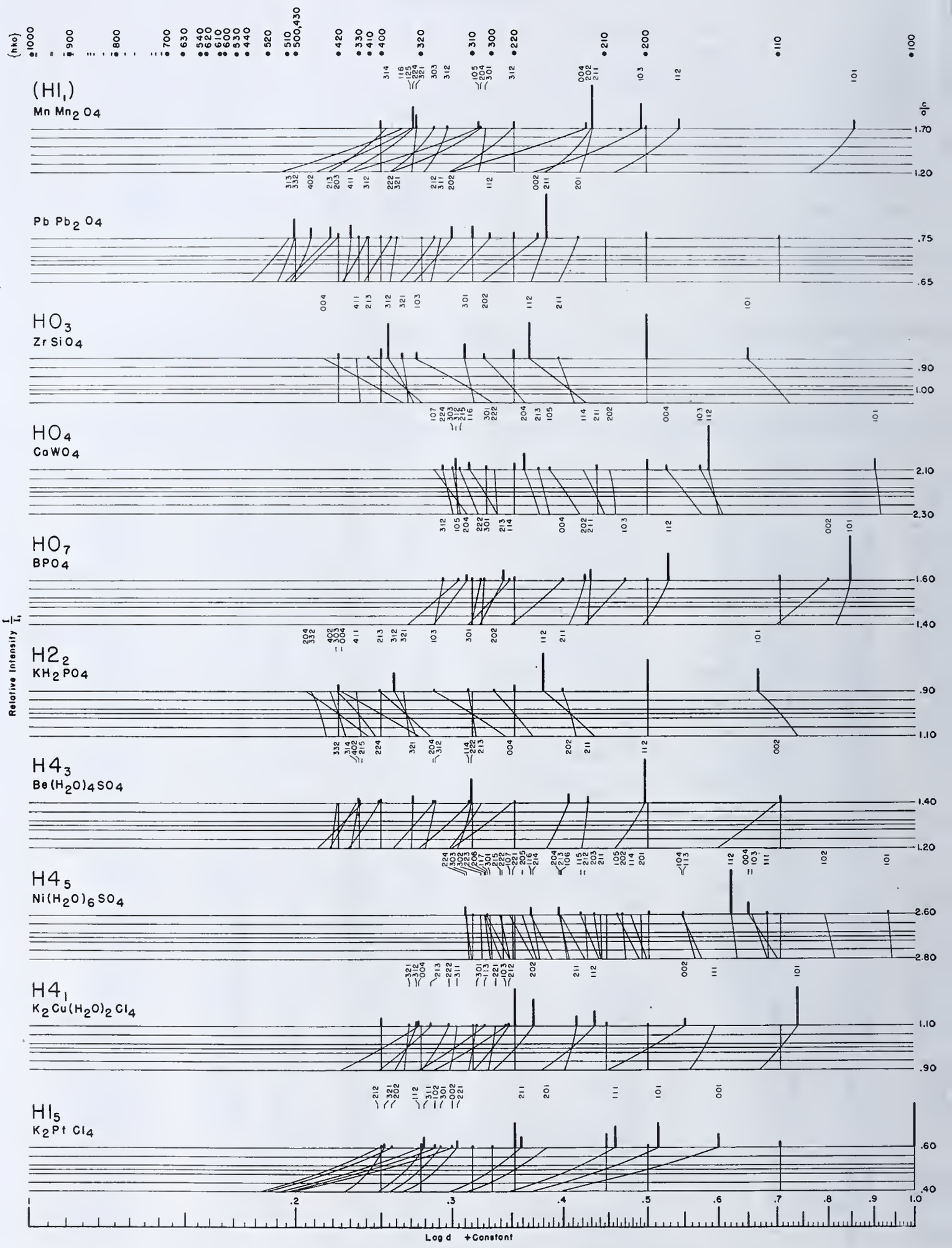

Figure 3. Representative Diffraction Patterns of Tetragonal Structures

Table IV (*Continued*)

$\frac{c}{a}$	a, kX	c, kX	Type	Substance
0.770	9.12	7.02	C^2_{4h}-P4$_2$/m	$C(COOCH_3)_4$
0.773	13.2	10.2	...	$LaAl_4$
0.780	10.45	8.14	...	$C_2(CH_3)_4Br_2$
0.784	8.87	6.95	...	$N(C_2H_5)_4I$
0.787	(4.79)	(3.77)	C4	TeO_2
0.803	9.29	7.46	C^3_{4h}-P4/n	PCl_5
0.806	6.052	4.878	C16	Al_2Cu
0.814	6.52	5.31	C16	Sn_2Fe
0.818	6.647	5.434	C16	Sn_2Mn
0.821	15.6	12.8	...	2-Hydroxy-10-methoxy-1,2,3,4,5,6,7,8,13,14,-15-dodecahydrochrysene
0.825	3.89	3.21	L10	NiZn
0.830	11.2	9.3	D^4_{2d}-P$\bar{4}2_1c$	$Si[SC(CH_3)_3]_4$
0.830	11.2	9.3	D^4_{2d}-P$\bar{4}2_1c$	$Ge[SC(CH_3)_3]_4$
0.830	11.2	9.3	D^4_{2d}-P$\bar{4}2_1c$	$Sn[SC(CH_3)_3]_4$
0.832	5.099	4.240	C16	Fe_2B
0.838	11.1	9.3	...	$(CH_3)_2CHS-Si[SC(CH_3)_3]_3$
0.841	5.006	4.212	C16	Co_2B
0.846	5.899	4.991	C16	Ge_2Fe
0.847	9.76	8.27	$NaBaPO_4$	$NaBaPO_4$
0.848	9.84	8.34	$NaBaPO_4$	$KBaPO_4$
0.851	4.980	4.236	C16	Ni_2B
0.852	6.835	5.821	C16	Pb_2Pd
0.857	6.348	5.441	C16	Sn_2Co
0.857	9.25	7.93	$NaBaPO_4$	$NaSrPO_4$
0.859	9.50	8.16	$NaBaPO_4$	$KSrPO_4$
0.867	7.13	6.18	HO_3	YVO_4
0.871	5.74	5.00	GO_{9-}	NH_4NO_3–II (357–398° K.)
0.873	8.02	7.00	B37	TlSe
0.874	7.25	6.34	HO_3	$CaCrO_4$
0.880	(6.32)	(5.56)	($E6_2$)	$SrO_2.8H_2O$
0.880	6.651	5.853	C16	Pb_2Rh
0.880	(11.36)	(9.96)	...	Ag_3Ca
0.888	6.87	6.10	HO_3	YPO_4
0.895	4.96	4.44	...	~ZrH_2
0.901	6.58	5.93	HO_3	$ZrSiO_4$
0.904	6.13	5.54	D^7_{4h}-P4/nmm	$CuB_2O_4.CuCl_2.4H_2O$, bandylite
0.906	(6.41)	(5.81)	($E6_1$)	$Sr(OH)_2.8H_2O$
0.910	6.89	6.27	HO_3	$YAsO_4$
0.917	5.83	5.35	...	~$MnBi_2$
0.921	9.69	8.92	$F1_1$	$Hg(CN)_2$
0.930	4.85	4.51	...	$PbIn_{2-3}$
0.933	8.48	7.91	C^5_{4h}-I4/m	$AgClO_3$
0.934	10.58	9.88	...	$(Ca,Na)_2Be(Si,Al)_2-(O,F)_7$, meliphanite
0.935	3.98	3.72	L10	AuCu
0.936	3.774	3.533	A6	γ-Mn
0.936	7.61	7.12	$H2_2$	KH_2AsO_4
0.937	5.83	5.46	C^1_{4v}-P4mm	$2Pb(OH)_2.CuCl_2$, diaboleite
0.938	7.43	6.97	$H2_2$	KH_2PO_4
0.941	8.59	8.08	...	$AgBrO_3$
0.948	(3.85)	(3.65)	...	$W_{12}O_{32}(OH)_2$
0.952	3.76	3.58	A6	95Mn.5Cu
0.960	3.77	3.62	...	96Mn.4Pd
0.962	3.767	3.624	A6	89Mn.11Cu
0.962	4.18	4.02	...	~70Mo-30N
0.966	3.85	3.72	L10	FePd
0.967	3.66	3.54	L10	NiMn
0.971	4.20	4.08	...	62Mn.38N
0.974	8.12	7.91	$J1_{11}$	$AgSb(OH)_6$
0.975	8.00	7.80	...	*trans*-$Pd(NH_3)_2Cl_2$
0.976	3.77	3.68	...	92Mn.8N
0.977	8.7	8.5	...	$Pd(NH_3)_2I_2$
0.981	3.764	3.693	A6	79Mn.21Cu
0.984	8.01	7.88	$J1_{11}$	$NaSb(OH)_6$
0.986	(3.61)	3.56	...	Ni_4Mo
0.998	3.752	3.744	A6	66Mn.34Cu
1.00	6.34	6.34	($E3_1$)	Ag_2HgI_4
1.003	7.52	7.54	$H2_2$	$NH_4H_2PO_4$
1.005	7.70	7.74	$H2_2$	$NH_4H_2AsO_4$
1.01	3.99	4.02	$E2_1$	$BaTiO_3$
1.01	4.96	5.03	(L12)	$SrPb_3$
1.01	(6.08)	(6.14)	($E3_1$)	Cu_2HgI_4
1.02	5.07	5.16	...	ZrO_2 (<1273° K.)
1.02	6.29	6.42	C^1_4-P4	$Pt(NH_3)_4PtCl_4$
1.02	7.81	8.00	$H4_1$	$Rb_2CuCl_4.2H_2O$
1.03	6.37	6.58	B34	(Pd, Pt, Ni)S
1.04	5.79	6.00	...	~Ni_2Sb
1.04	6.35	6.60	B34	PdS
1.04	10.29	10.55	...	$Mg(ClO_2)_2.6H_2O$
1.04	10.64	11.07	D^{10}_4-I4$_12$	Cr_2Ni
1.05	7.58	7.96	$H4_1$	$(NH_4)_2CuCl_4.2H_2O$
1.05	7.9	8.3	$H4_1$	$(NH_4)_2CuBr_4.2H_2O$
1.05	12.95	13.65	C^6_{4h}-I4$_1$/a	$KAlSi_2O_6$, leucite
1.06	2.84	3.01	...	α-Martensite
1.06	3.89	4.13	($E2_1$)	$PbTiO_3$
1.06	7.45	7.88	$H4_1$	$K_2CuCl_4.2H_2O$
1.06	22.0	23.3	...	$Al_2C_{12}O_{12}.18H_2O$, mellite
1.08	4.585	4.937	A6	In
1.09	7.50	8.16	$H4_1$	$(NH_4)_2FeCl_4.2H_2O$
1.09	8.13	8.83	Phosgenite	$Pb_2Cl_2CO_3$
1.09	8.34	9.06	Phosgenite	$Pb_2Br_2CO_3$
1.13	6.70	7.60	K_3CrO_8	K_3CrO_8
1.13	7.37	8.34	K_3CrO_8	Cs_3TaO_8
1.14	(5.33)	(6.08)	...	$AgFO_3$
1.14	7.05	8.05	K_2CrO_8	Rb_3TaO_6
1.14	15.84	18.01	$J3_1$	$K_2TlCl_6.2H_2O$
1.15	6.27	7.22	C11	CsO_2
1.15	16.95	19.45	$J3_1$	$Rb_3TlBr_6.8/7H_2O$
1.16	5.99	7.02	C11	RbO_2
1.16	6.07	7.03	$F5_2$	KNCO
1.16	6.09	7.06	$F5_2$	KN_3
1.16	6.78	7.86	K_3CrO_8	K_3CbO_8
1.16	6.78	7.88	K_3CrO_8	K_3TaO_8
1.17	2.75	3.21	L10	NiZn
1.17	6.36	7.41	$F5_2$	RbN_3
1.18	5.70	6.73	C11	KO_2
1.20	...	...	C11	UC_2
1.20	4.28	5.13	OB21	CH_3NH_3Cl
1.20	5.67	6.81	$F5_2$	KFHF
1.21–1.31	4.05–3.98	4.90–5.10	D^7_{4h}-P4/nmm	PbO-Bi_2O_3
1.21	6.98	8.42	$H5_{10}$	$Ca(UO_2)_2(PO_4)_2.6-1/2H_2O$
1.22	3.55	4.33	B10	LiOH
1.25	6.99	8.75	$J1_5$	$K_2OsO_2Cl_4$
1.26	3.36	4.25	...	α-LiBi
1.26	3.98	5.01	B10	PbO
1.26	12.43	15.6	...	$Ca_4NaAl_3Si_5O_{19}$, sarcolite
1.27	3.80	4.81	B10	SnO
1.28	(4.14)	(5.28)	C11	ThC_2
1.28	8.81	11.27	...	$C_2(CH_3)_2Br_4$
1.28	11.70	14.95	...	$Fe_2(TeO_3)_3.xH_2O$, mackayite
1.29	8.97	11.55	D^2_{4h}-P4/mcc	$Sr(OH)_2.8H_2O$
1.32	2.669	3.533	A6	γ-Mn
1.32	2.78	3.66	...	Ni-N
1.32	2.81	3.72	L10	AuCu
1.32	7.22	9.53	...	C_4H_4S (~100° K.), thiophene
1.33	4.73	6.29	...	$5PbCrO_4.3PbMoO_4.-10PbSO_4$
1.35	2.66	3.58	A6	95Mn.5Cu
1.35	3.24	4.38	NaBi	MgIn
1.35	7.38	9.96	...	$(Ca,Na)_2BeSi_2(O,OH,F)_7$, leucophanite
1.36	2.66	3.62	A6	89Mn.11Cu
1.36	7.57	10.28	D^{19}_{4h}-I4/amd	$Ba(CH_2COO)_2$
1.37	2.59	3.54	L10	NiMn
1.37	2.72	3.72	L10	FePd
1.39	2.66	3.69	A6	79Mn.21Cu
1.39	3.46	4.80	NaBi	NaBi
1.40	4.96	6.92	C30	SiO_2
1.40	8.75	12.28	$D5_9$	Cd_3P_2
1.41	2.65	3.74	A6	66Mn.34Cu
1.41	5.00	7.06	C30	$AlPO_4$
1.41	5.48	7.74	...	Li_2O_2
1.41	8.10	11.39	...	Ni_9Sb_4
1.41	8.10	11.45	$D5_9$	Zn_3P_2
1.41	8.32	11.76	$D5_9$	Zn_3As_2
1.41	8.95	12.65	$D5_9$	Cd_3As_2
1.42	7.88	11.19	$OJ1_1$	$[N(CH_3)_4]_2SiF_6$
1.43	12.83	18.38	D^6_{4h}-P4/mnc	$B_2O_3.24WO_3.66H_2O$
1.43	12.98	18.52	...	$H_4SiW_{12}O_{40}.31H_2O$
1.44	6.09	8.76	$O4_{12}$	$C(CH_2OH)_4$
1.44	12.80	18.40	...	$(NH_4)_5BW_{12}O_{40}.26H_2O$
1.45	7.49	10.87	$K7_6$	$Cs_2AuAuCl_6$
1.45	9.50	13.81	D^1_{4h}-P4/mmm	$CuCl.3SC(NH_2)_2$
1.46	5.548	8.093	DO_{22}	$TiGa_3$
1.46	(7.74)	(11.31)	HO_3	$Y(Cb, Ta)O_4$
1.46	(7.76)	(11.32)	HO_3	$YCbO_4$
1.47	3.77	5.52	B10	FeSe
1.47	(7.75)	(11.41)	HO_3	$YTaO_4$
1.48	5.85	8.68	($H1_1$)	$CuFe_2O_4$
1.48	7.01	10.36	$K7_8$	$Na_5Al_3F_{14}$
1.49	6.65	9.91	...	Na_2O_2
1.49	7.38	11.01	$K7_6$	$Cs_2AgAuCl_6$
1.50	6.97	10.43	$J1_9$	$AgCo(NH_3)_2(NO_2)_4$
1.51	4.14	6.25	...	$Pb(ClO_2)_2$
1.51	4.85	7.33	OB21	$C_3H_7NH_3I$
1.52	3.24	4.94	A6	In
1.52	4.459	6.796	HO_7	$BAsO_4$
1.53	3.992	6.091	C38	Cu_2Sb
1.53	4.332	6.640	HO_7	BPO_4
1.55	5.605	8.712	DO_{22}	$ZrGa_3$
1.56	5.334	8.305	DO_{22}	VAl_3
1.56	9.07	14.12	C^6_{4v}-P4cc	$[N(CH_3)_2(C_2H_5)_2]_2SnCl_6$
1.57	5.17	8.12	...	~Fe_2Ti
1.57	5.422	8.536	DO_{22}	$TaAl_3$
1.58	4.48	7.10	OB21	$C_3H_7NH_3Cl$
1.58	5.425	8.579	DO_{22}	$TiAl_3$
1.58	5.427	8.584	DO_{22}	$CbAl_3$
1.58	6.20	9.82	($H1_1$)	$CaIn_2O_4$
1.58	9.18	14.47	$K3_1$	Cs_3CoCl_5
1.59	5.74	9.15	($H1_1$)	$ZnMn_2O_4$
1.61	4.08	6.56	C38	Mn_2Sb
1.61	4.39	7.05	C11	BaC_2
1.61	4.57	7.36	OB21	$C_3H_7NH_3Br$
1.63	6.12	9.87	($H1_1$)	$CdIn_2O_4$
1.61	8.7	14.0	$K3_1$	Rb_3CoCl_5
1.61	4.11	6.68	C11	SrC_2
1.63	15.0	24.4	D^{17}_{4h}-I4/mmm	~$PbCl_2.Cu(OH)_2$, cumengeite
1.64	5.75	9.42	($H1_1$)	$MnMn_2O_4$
1.65	3.627	5.973	C38	Fe_2As

(*Continued on page 93*)

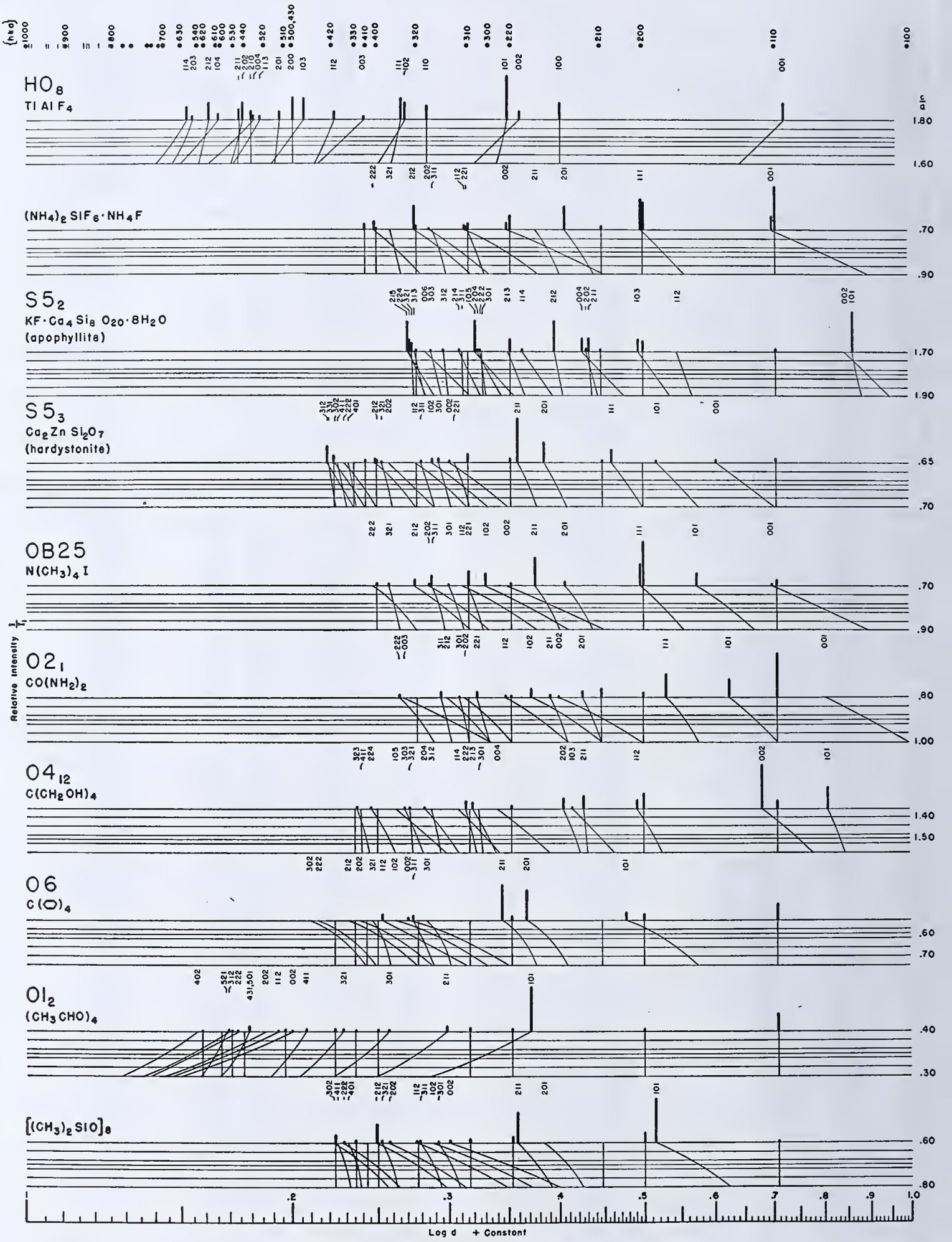

Figure 4.. Representative Diffraction Patterns of Tetragonal Structures

Table IV (*Continued*)

$\frac{c}{a}$	a, kX	c, kX	Type	Substance
1.65	3.82	6.30	C11	NdC_2
1.65	3.87	6.37	C11	CaC_2
1.65	4.38	7.22	EO_1	BaFCl
1.66	3.85	6.38	C11	PrC_2
1.67	3.54	5.91	C11	CaO_2
1.67	3.75	6.28	C11	SmC_2
1.67	3.76	6.27	C38	Mn_2As
1.67	3.87	6.48	C11	CeC_2
1.67	3.92	6.55	C11	LaC_2
1.68	4.10	6.88	EO_1	SrFCl
1.68	9.93	16.70	...	*l*-$Co(NH_2.CH_2.CH_2.NH_2)_3Br_3.H_2O$
1.69	5.83	9.88	D_{4h}^{19}-I4/*amd*	$6CuO.Cu_2O$, paramelaconite
1.71	4.65	7.93	EO_1	BAFI
1.71	8.39	14.34	$K3_4$	$NH_4Pb_2Br_5$
1.72	5.09	8.76	OB20	CH_3NH_3Br
1.72	8.41	14.5	$K3_4$	$RbPb_2Br_5$
1.73	3.55	6.14	HO_6	$KAlF_4$
1.73	3.62	6.26	HO_6	$RbAlF_4$
1.73	8.14	14.1	$K3_4$	KPb_2Br_5
1.74	3.03	5.26	B17	PdO
1.75	3.613	6.333	C38	Cr_2As
1.75	5.11	8.96	OB20	CH_3NH_3I
1.76	3.03	5.32	B17	PtO
1.76	3.47	6.10	B17	PtS
1.76	3.61	6.37	HO_6	$TlAlF_4$
1.76	3.89	6.83	EO_1	CaFCl
1.76	4.09	7.21	EO_1	PbFCl
1.76	9.00	15.84	$S5_2$	$KCa_4Si_8O_{20}F.8H_2O$, apophyllite
1.77	3.59	6.35	HO_8	NH_4AlF_4
1.79	3.78	6.77	C11	BaO_2
1.79	14.38	25.80	D_4^{10}-I4$_1$2	$KUO_2(CH_3COO)_3$
1.81	5.72	10.37	...	α-$Pt(NH_3)_2Cl_4$
1.82	4.18	7.59	EO_1	PbFBr
1.82	5.66	10.30	$E1_1$	$AgFeS_2$
1.83	4.16	7.61	D_{4h}^{10}-P4/*mcm*	NH_4CN
1.84	10.7	19.7	...	$(C_2H_5)_3As.AgI$
1.85	3.55	6.55	C11	SrO_2
1.89	3.89	7.37	EO_1	BiOCl
1.89	4.19	7.94	$E2_8$	NH_4HgCl_3
1.90	2.69	5.10	...	$FeSi_2$
1.94	4.70	9.10	$FeTa_2O_6$	$NiTa_2O_6$
1.94	4.71	9.12	$FeTa_2O_6$	$Fe(Cb,Ta)_2O_6$, mossite
1.94	4.73	9.16	$FeTa_2O_6$	$CoTa_2O_6$
1.95	4.70	9.18	$FeTa_2O_6$	$MgTa_2O_6$
1.95	4.71	9.18	$FeTa_2O_6$	$FeTa_2O_6$, tapiolite
1.95	7.80	15.23	...	$Pb(Cl,OH)_2.4PbO.2Fe_2O_3$, hämatophanite
1.97	(4.28)	(8.42)	C11	KHC_2
1.97	5.26	10.37	$E1_1$	$CuFeS_2$, chalcopyrite
1.97	5.46	10.73	$H2_8$	Cu_2FeSnS_4, stannite
1.98	13.99	27.70	D_4^{10}-I4$_1$2	$KUO_2(CH_3COO)_3$
1.99	4.02	8.02	...	H_2O_2
1.99	9.50	18.93	D_{4h}^{20}-I4/*acd*	$3Mn_2O_3.MnSiO_3$, braunite
2.00	13.79	27.60	D_4^{10}-I4$_1$2	$NH_4UO_2(CH_3COO)_3$
2.03	15.4	31.2	...	$Pb_3Cu_4Cl_{10}O_4.6H_2O$, pseudoboleite
2.06	2.83	5.82	...	~CuGa$_2$
2.06	3.92	8.09	DO_{22}	$TiGa_3$
2.07	3.92	8.11	EO_1	BiOBr
2.08	5.42	11.3	D_{2d}^{12}-I$\bar{4}$2*d*	$(Bi,W)_8$-nO_{12}, russellite
2.10	(3.89)	(8.17)	C11	$NaHC_2$
2.14	7.50	16.05	D_{4h}^{8}-P4/*ncc*	$BaFeSi_4O_{10}$, gillespite
2.15	5.94	12.80	HO_4	NH_4IO_4
2.16	12.98	28.10	$O4_{11}$	$AgUO_2(CH_3COO)_3$.-xH_2O
2.17	5.15	11.17	HO_4	$CdMoO_4$
2.17	5.24	11.38	HO_4	$CaWO_4$
2.17	5.35	11.63	HO_4	$NaLa(WO_4)_2$
2.18	5.32	11.59	HO_4	$NaCe(WO_4)_2$
2.18	5.32	11.60	$La_2(MoO_4)_3$	$Pr_2(MoO_4)_3$
2.18	5.34	11.63	HO_4	$LiLa(WO_4)_2$
2.19	5.23	11.44	HO_4	$CaMoO_4$
2.19	5.27	11.55	HO_4	$NaBi(MoO_4)_2$
2.19	5.29	11.58	$La_2(MoO_4)_3$	$Nd_2(MoO_4)_3$
2.19	5.36	11.72	HO_4	$NaReO_4$
2.20	3.77	8.31	DO_{22}	VAl_3
2.20	3.96	8.71	DO_{22}	$ZrGa_3$
2.20	5.23	11.50	HO_4	$LiBi(MoO_4)_2$
2.20	5.31	11.67	HO_4	$LiLa(MoO_4)_2$
2.20	5.33	11.70	HO_4	$NaLa(MoO_4)_2$
2.20	5.40	11.90	HO_4	$SrWO_4$
2.20	5.75	12.63	HO_4	KIO_4
2.20	5.87	12.94	HO_4	$RbIO_4$
2.20	5.87	12.94	HO_4	NH_4ReO_4
2.21	5.33	11.78	$La_2(MoO_4)_3$	$Ce_2(MoO_4)_3$
2.21	5.35	11.84	$La_2(MoO_4)_3$	$La_2(MoO_4)_3$
2.21	5.44	12.01	HO_4	$PbWO_4$
2.21	5.44	12.03	HO_4	$KLa(WO_4)_2$
2.22	5.38	11.92	HO_4	$KBi(MoO_4)_2$
2.22	5.39	11.94	HO_4	$KCe(WO_4)_2$
2.22	5.62	12.50	HO_4	$KReO_4$
2.23	3.83	8.54	DO_{22}	$TaAl_3$
2.23	5.22	11.62	$La_2(MoO_4)_3$	$Sn_2(MoO_4)_3$
2.23	5.35	11.92	HO_4	$AgReO_4$
2.23	5.37	11.96	HO_4	$SrMoO_4$
2.23	5.41	12.08	HO_4	$PbMoO_4$
2.23	5.42	12.11	HO_4	$KLa(MoO_4)_2$
2.24	3.836	8.579	DO_{22}	$TiAl_3$
2.24	3.837	8.584	DO_{22}	$CbAl_3$
2.24	5.32	11.93	HO_4	$NaIO_4$
2.24	5.37	12.01	HO_4	$AgIO_4$
2.26	5.62	12.70	HO_4	$BaWO_4$
2.27	5.80	13.17	HO_4	$RbReO_4$
2.28	4.01	9.14	EO_1	BiOI
2.29	5.56	12.76	HO_4	$BaMoO_4$
2.30	5.08	11.69	HO_4	$BiAsO_4$
2.31	5.76	13.33	HO_4	β-$TlReO_4$ (400° K.)
2.32	5.65	13.08	HO_4	$KOsO_3N$
2.36	4.92	11.62	$D3_1$	Hg_2I_2
2.36	5.46	12.89	HO_4	$KCrO_3F$
2.36	10.09	23.85	...	$C_{28}H_{36}N_4$, acetonylpyrrol
2.39	4.65	11.10	$D3_1$	Hg_2Br_2
2.40	10.0	24.0	D_{4h}^{19}-I4/*amd*	*cis*-$[Pt(NH_3)(C_2H_4)Cl_2]_2$
2.42	12.5	30.25	...	$6Pb(S,Tl)_2.AuTl_2$, nagyagite
2.44	4.46	10.89	$D3_1$	Hg_2Cl_2
2.45	3.21	7.88	C20	WSi_2
2.46	3.20	7.86	C20	$MoSi_2$
2.46	4.53	11.14	$D1_2$	Al_4Ba
2.47	6.29	15.55	...	$(CH_2CO)_2NI$
2.48	4.45	11.04	$D1_8$	Al_4Sr
2.51	3.75	9.43	C5	TiO_2
2.52	5.61	14.13	HO_4	$CsSO_3F$
2.53	5.72	14.50	HO_4	$CsCrO_3F$
2.54	4.35	11.07	$D1_2$	Al_4Ca
2.68	7.04	18.88	.	$CaNa_4Al_{12}(PO_4)_8(OH)_{18}.6H_2O$, wardite
2.80	6.40	17.95	D_4^3-P4$_1$2	$C_5H_4O_4N_4$, *l*-spiro-5,5-dihydantoin
2.80	6.95	19.45	...	$[(NH_2)_2CNH]_2.H_2CO_3$
2.81	4.78	13.43	$Tl(CH_3)_2I$	$Tl(CH_3)_2I$
2.82	7.03	19.8	...	$2,4,6(C_5H_2)I(NO_2)_3$
2.82	7.25	20.47	...	$C_5H_4[1,2](COC_2H_5)_2$
2.83	4.36	12.36	C13	HgI_2
2.88	2.998	8.630	C48	Cr_2Al
2.91	7.05	20.5	D_{4h}^{17}-I4/*mmm*	$Cu(UO_2)_2(PO_4)_2.8H_2O$ torberite
2.95	6.99	20.63	$H5_9$	$Ca(UO_2)_2(PO_4)_2.10^1/_2H_2O$
2.95	5.18	15.3	OB20	$C_4H_9NH_3I$
2.96	5.02	14.85	OB20	$C_4H_9NH_3Cl$
2.98	3.66	10.9	$D3_1$	Hg_2F_2
3.02	5.96	17.99	...	$C_2H_4(NH_2)_2.H_2SO_4$
3.03	5.02	15.23	OB20	$C_4H_9NH_3Br$
3.08	4.47	13.78	$Tl(CH_3)_2I$	$Tl(CH_3)_2Br$
3.13	3.840	12.03	D_{4h}^{17}-I4/*mmm*	$LiBi_3O_4Cl_2$
3.13	3.877	12.13	$LiBi_3O_4Cl_2$	$NaBi_3O_4Cl_2$
3.16	5.513	17.422	...	$MnSi_2$
3.20	3.925	12.55	$LiBi_3O_4Cl_2$	$NaBi_3O_4Br_2$
3.20	3.943	12.62	$LiBi_3O_4Cl_2$	$Cd_2Bi_2O_4Br_2$
3.22	3.876	12.47	$LiBi_3O_4Cl_2$	$LiBi_3O_4Br_2$
3.27	4.29	14.02	$Tl(CH_3)_2I$	$Tl(CH_3)_2Cl$
3.33	5.01	16.70	OB20	$C_5H_{11}NH_3Cl$
3.34	3.97	13.24	$LiBi_3O_4Cl_2$	$Cd_2Bi_2O_4I_2$
3.34	3.990	13.31	$LiBi_3O_4Cl_2$	$NaBi_3O_4I_2$
3.35	3.941	13.19	$LiBi_3O_4Cl_2$	$LiBi_3O_4I_2$
3.35	5.16	17.3	OB20	$C_5H_{11}NH_3I$
3.40	5.00	17.0	OB20	$C_5H_{11}NH_3Br$
3.47	4.13	14.35	D_{4h}^{19}-I4/*amd*	$ThSi_2$
3.68	5.07	18.65	ZnP_2	ZnP_2
3.73	5.28	19.70	ZnP_2	CdP_2
3.78	5.16	19.50	OB20	$C_6H_{13}NH_3I$
3.91	4.09	15.99	...	La_2MoO_6
3.93	4.98	19.55	OB20	$C_6H_{13}NH_3Cl$
4.02	4.93	19.8	OB20	$C_6H_{13}NH_3Br$
4.03	15.4	62.0	...	$Pb_9Cu_8Ag_3Cl_{21}O_8.9H_2O$, boleite
4.15	5.18	21.5	OB20	$C_7H_{15}NH_3I$
4.25	4.96	21.1	OB20	$C_7H_{15}NH_3Cl$
4.32	4.003	17.29	DO_{23}	$ZrAl_3$
4.58	5.18	23.7	OB20	$C_8H_{17}NH_3I$
4.59	7.32	33.6	D_4^4-P4$_1$2$_1$	β-methyl *d*-glucoside
5.42	5.18	28.1	OB20	$C_{10}H_{21}NH_3I$
5.76	3.78	21.77	...	Beyerite
5.88	5.18	30.46	OB20	$C_{11}H_{23}NH_3I$
6.03	5.18	31.24	OB20	$C_{12}H_{25}NH_3I$
6.74	5.7	38.4	...	$C_{14}H_6O_2[2,7](NO_2)_2$ 2,7-dinitroanthraquinone

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Application of Colorimetry to the Analysis of Corrosion-Resistant Steels

Photometric Determination of Copper

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Photometric measurement of the cuprammonium ion, $Cu(NH_3)_4^{++}$, is best made at 580 millimicrons, where the effects of ammonia concentration and other ions that may be present are negligible. Interference at 620 millimicrons, the region of maximum sensitivity, is significant. A rapid procedure, applicable to low-alloy and corrosion-resistant steels, is given and data are presented in support of the validity of the method.

DURING an investigation of methods for the determination of low percentages of residual elements in corrosion-resistant steels (*1, 2, 10, 14*), a rapid photometric determination of copper was developed which is more easily adapted to routine use than the usual gravimetric α-benzoinoxime procedure (*6*) or that in which copper is separated as thiocyanate and titrated iodometrically (*7*).

The colorimetric determination of copper by conversion to the cuprammonium ion, $Cu(NH_3)_4^{++}$, has been used for a number of years (*5, 13, 15*). The principal objection to the method is that the color is a function of the ammonia concentration which, because of the volatile nature of the reagent, is not always easily controlled.

Mehlig, in the analysis of copper ores (*8*), has overcome the difficulty by destroying the color of the ammine with cyanide, estimating the copper by the difference in spectrophotometric transmission before and after the cyanide addition. Yoe and Barton (*16*) suggest the use of less volatile triethanolamine for the photoelectric measurement of the cuprammonium color. Among organic reagents suggested for the colorimetric determination of copper, sodium diethyldithiocarbamate (*3*), dithizone (*4*), and 1,10-phenanthroline (*11*) have been found applicable.

It was believed, however, that the full possibilities of the use of ammonia for the photometric determination of copper had not been explored and an investigation of conditions was begun.

SPECTROPHOTOMETRIC INVESTIGATION

In spectrophotometric studies previously made (*9, 16*) it has been found that both absorption by the copper-ammonia complex and wave length of maximum absorption increase with the ammonia concentration. At the usual concentration of about 3 *N*, maximum absorption is at 620 millimicrons. From one of these studies (*16*), however, it was observed that the transmissions of two solutions containing 100 p.p.m. of copper with ammonia concentrations of 0.4 *M* and 2.5 *M*, respectively, coincided at about 560 millimicrons. From this it appeared that if there were a spectral band at which the ammonia concentration had a negligible effect, the absorption of the copper-ammonia complex at such a wave length could be conveniently utilized for the accurate photometric determination of copper. Accordingly, four solutions were prepared, each containing 2 mg. of copper (as the sulfate) and an excess of ammonium hydroxide (0.90 specific gravity) varying from 5 to 20 ml. The solutions were diluted to 100 ml. in flasks which were stoppered to prevent escape of ammonia, then rapidly transferred to absorption cells and transmissions immediately measured by a General Electric recording spectrophotometer set for a spectral band width of 10 millimicrons. The curves obtained (Figure 1) intersected at approximately 580 millimicrons, but, in agreement with the results of the previous investigations, there were considerable differences in the region of maximum absorption. The spectrophotometric transmission measurement was repeated for two series of solutions containing 5 and 10 mg. of copper, respectively, with analogous results (Figure 1).

The absorptions of the solutions were then measured with a Klett-Summerson photoelectric colorimeter, using a filter with maximum transmission at 580 millimicrons and a 4-cm. glass absorption cell. The galvanometer scale readings, which are logarithmic on this instrument, were found to be almost directly proportional to the copper content. The difference in colorimeter readings between solutions containing the same amount of copper but different ammonia concentrations was negligible. Measurement of the same solutions using a filter with maximum transmission at 620 millimicrons, however, gave readings which decreased proportionately with a decrease in ammonia concentration; a solution containing the equivalent of 0.20% of copper with a 20-ml. excess showed only 0.16% present with a 5-ml. excess.

Müller (*12*) found that Beer's law holds for the copper-ammonia complex up to concentrations of about 1 gram of copper per liter, using a filter transmitting in the region of maximum sensitivity. The author's series of measurements at 580 millimicrons, however, shows the curve of concentration *vs.* log transmission to deviate slightly from linearity at concentrations above 60 mg. per liter.

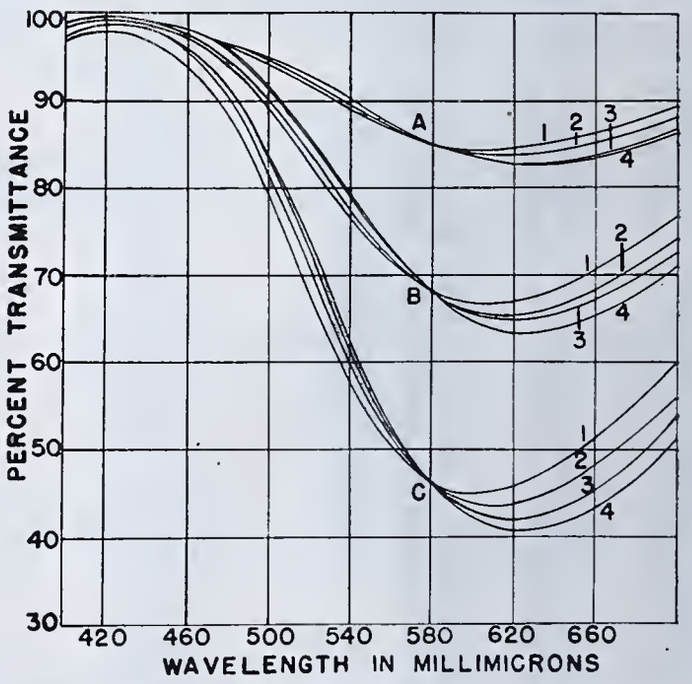

Figure 1. Transmittance of Ammoniacal Copper Solutions (4-Cm. Cell)

Set No.	Copper Concentration *Mg./100 ml.*	Curve No.	NH_4OH Excess *Ml.*
A	2	1	5
B	5	2	10
C	10	3	15
		4	20

Table I. Determination of Copper in Steel

Sample	Type of Steel	Copper Present %	Copper Found %
N.B.S. 19d	Plain carbon	0.158	0.16
N.B.S. 34a	Plain carbon	0.222	0.22
N.B.S. 35a	Plain carbon	0.267	0.27
N.B.S. 73a	14% Cr	0.080	0.08
N.B.S. 101b	18% Cr–9% Ni	0.16[a]	0.16
N.B.S. 121a	18% Cr–10% Ni 0.4% Ti	0.08[a]	0.06
N.B.S. 133	14% Cr–0.66% Mo	0.061	0.06
Lab. No. T-1757	20% Cr–10% Ni 4.1% Mn, 0.22% Co	0.30[b]	0.31
Lab. No. T-2284	27% Cr–22% Ni 1.8% Mn	0.13[b]	0.13
Lab. No. T-2285	21% Cr–11% Ni 4.6% Mn, 0.23% Cb	0.25[b]	0.25

[a] Provisional value.
[b] Determined by CuCNS–$Na_2S_2O_3$–KI titration method.

PRELIMINARY SEPARATION

It was found that the necessary preliminary separation of the copper is most rapidly and conveniently made by precipitating as the sulfide with thiosulfate (*7*). The copper precipitate is ordinarily accompanied by some iron, manganese, and free sulfur. Silica may also separate out during the dissolution of the sample, but can easily be eliminated by a few drops of hydrofluoric acid. Iron is precipitated upon the subsequent addition of ammonia and may be filtered off prior to the colorimetric estimation. Free sulfur and small amounts of manganese are removed with the iron; larger quantities of manganese, that may be coprecipitated with the copper sulfide from special steels containing as high as 4 or 5% of this element, frequently escape immediate precipitation with the ammonia but come down slowly upon standing. In this case, quantitative separation may be made by the addition of an oxidant, such as ammonium persulfate, to the ammoniacal solution.

In the proposed method, the impure copper sulfide precipitate is returned to the original flask after filtration and decomposed by the use of a mixture of nitric and perchloric acids, instead of being ignited to mixed oxides and redissolved in nitric acid. Perchlorates do not interfere with the copper-ammonia color (*9*); however, a minimum of perchloric acid is used to avoid formation of slightly soluble ammonium perchlorate crystals. The dissolution of corrosion-resistant steels, especially those of the 25% Cr–20% Ni types, frequently proceeds slowly in sulfuric acid alone. Accordingly, a mixture of hydrochloric and sulfuric acids is used to dissolve the sample.

APPARATUS AND REAGENTS

Klett-Summerson Photoelectric Colorimeter, Research model.

4-Cm. Glass Absorption Cell.

Glass Filter, transmission maximum at 580 millimicrons.

Solvent Acid Mixture. Add 250 ml. of concentrated hydrochloric acid to a mixture of 600 ml. of water and 150 ml. of concentrated sulfuric acid.

Sodium Thiosulfate Solution, 50%. Dissolve 500 grams of sodium thiosulfate in 500 ml. of water.

Nitric-Perchloric Acid Mixture. Mix 250 ml. of concentrated nitric acid and 80 ml. of perchloric acid (70 to 72%).

Ammonium Nitrate Solution, 1%. Dissolve 10 grams of ammonium nitrate in 1 liter of water.

PROCEDURE

Transfer 5 grams of sample to a covered 500-ml. Erlenmeyer flask and dissolve by warming gently with 100 ml. of solvent acid mixture. When dissolution is complete, remove the cover glass, add a few drops of hydrofluoric acid (48%), and boil for several minutes. Dilute to about 300 ml., heat to gentle boiling, and carefully add 20 ml. of sodium thiosulfate solution in 3- to 5-ml. increments. Continue the boiling until the precipitate is well coagulated (usually 15 to 20 minutes). Decant the hot solution through a coarse filter, rinse, and wash well with hot water.

Return the paper and precipitate to the original flask and add 35 ml. of nitric-perchloric acid mixture. Swirl the flask until the contents are thoroughly moistened by the acid mixture. Heat at a moderate temperature until the paper is decomposed and the sulfides are oxidized to yellow beads of free sulfur. Continue the digestion until dense fumes of perchloric acid are evolved, then allow to cool.

Add 35 ml. of water, neutralize with ammonium hydroxide, and add an excess of 2 to 3 ml. Heat to boiling. (If the steel is known to contain more than 1% of manganese, use an excess of 10 ml. of ammonium hydroxide, and add 2 grams of ammonium persulfate crystals to the boiling solution.) Continue the boiling for a minute or two, testing with a piece of litmus paper to ensure that the solution is still ammoniacal. Allow to cool to room temperature and filter through a coarse filter paper into a 100-ml. volumetric flask. Add 20 ml. of ammonium hydroxide to the filtrate. Rinse and wash with ammonium nitrate solution until the level in the flask just reaches the mark. Stopper, and mix well.

Set the zero point of the colorimeter with diluted ammonium hydroxide (1 to 4). Transfer a portion of the unknown solution to the absorption cell and obtain the colorimeter reading. Convert to percentage of copper by reference to a calibration curve prepared for the instrument by the use of portions of a standard copper nitrate solution.

ACCURACY AND PRECISION

Application of the method to the determination of copper in National Bureau of Standards and laboratory standard samples gave the results shown in Table I. The accuracy is comparable to that of other commonly used procedures. The precision of the method is good; the average deviations for N.B.S. samples 73a and 101b were about 0.002%.

CONCLUSION

The method is rapid—a set of 12 determinations can be completed in 2 hours after the samples have been dissolved. The speed of dissolution varies with the alloy composition and fineness of the sample; from 1 to 3 hours are required for corrosion-resistant steels, less for slightly alloyed steels.

Interference by other elements is negligible. Experiments have shown that in the analysis of corrosion-resistant steels no chromium or nickel is coprecipitated with the copper sulfide even when the amounts present run as high as 25% of each. However, small amounts of chromium, in the form of undecomposed carbides, may accompany the copper sulfide from special corrosion-resistant steels of high carbon content. The subsequent fuming with perchloric acid partially decomposes these carbides and oxidizes the chromium to chromic acid which passes into the filtrate with the copper-ammine. The author found that an ammoniacal copper solution containing 1000 parts of chromium (as chromate) per million showed an error of less than 0.002%—within the precision of the method. If desired, however, this slight interference may be completely eliminated by reducing the chromium with a few drops of hydrogen peroxide after the fuming with perchloric acid and removing as the insoluble hydroxide with the iron.

Although molybdate is included by Mehlig in a list of interfering anions (*9*), there was no measurable increase in absorption at 580 millimicrons upon the addition of 0.075 gram of molybdic acid (MoO_3) to several of the test solutions. This quantity of molybdate is equivalent to that formed from a steel containing 1% of molybdenum, assuming 100% coprecipitation of molybdenum with the copper.

Tungsten is occasionally present in corrosion-resistant steels in low residual amounts. It is, for the most part, removed during the course of the analysis; traces that may remain do not cause significant interference.

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Colorimetric Determination of Nitrites

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A General Electric recording spectrophotometer and a Beckman spectrophotometer were used to determine the effect of reagent concentration, order of addition of reagents, pH, temperature, light, nitrite concentration, and the presence of 68 diverse ions on the reddish purple coloration produced by 4-aminobenzenesulfonic acid (sulfanilic acid) and 1-aminonaphthalene (α-naphthylamine) in the presence of nitrous acid. Reliable determinations can be made in the range of 0.025 to 0.600 p.p.m. of nitrite ion in a 2-cm. cell. As little as 0.0005 p.p.m. of nitrite nitrogen can be determined by the use of 24-cm. Nessler tubes. Time must be allowed for complete diazotization before the coupling agent is added. Diazotization is carried out in strongly acidic solution at room temperature in diffuse light, and the system is buffered to pH 2.0 to 2.5 for coupling.

FOR decades small amounts of nitrite have been determined colorimetrically. This determination usually depends upon diazotization and coupling reactions. Nitrites react with primary aromatic amines in acidic solutions with the formation of diazonium salts which will couple with certain compounds to form intensely colored azo dyes.

Many procedures have been given for the diazotization of 4-aminobenzenesulfonic acid (sulfanilic acid) and coupling with 1-aminonaphthalene (α-naphthylamine) (*1*, *2*, *10*, *13*). Wallace and Neave (*18*) proposed the use of *N*,*N*-dimethyl-1-aminonaphthalene as the coupling agent. This has been used by some (*5*, *6*). Bratton and Marshall (*3*) investigated new coupling agents for diazotized 4-aminobenzenesulfonamide (sulfanilamide) and found *N*-(1-naphthyl)-ethylenediamine dihydrochloride most satisfactory. Shinn (*15*) and Kershaw and Chamberlain (*9*) made use of 4-aminobenzenesulfonamide and this coupling agent for the determination of nitrites.

This paper embodies the results of an investigation of the use of 4-aminobenzenesulfonic acid and 1-aminonaphthalene which is recommended by the American Public Health Association (*1*). The present investigation was undertaken to determine the sources of error in the method still officially recognized.

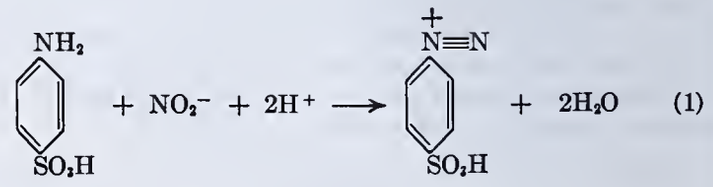
EXPERIMENTAL WORK

Apparatus and Solutions. Transmittancy measurements were made with a General Electric recording spectrophotometer operating with a spectral band width of 10 mμ, and with a Beckman spectrophotometer operating with a band width of approximately 1 mμ. Equivalent amounts of reagents were added to distilled water in the reference cell. All pH determinations were made with a glass electrode pH meter.

To prepare a stock solution of sodium nitrite (*14*) a solution containing about 1 gram of the salt in 100 ml. was aliquoted and analyzed by titration with potassium permanganate according to the U.S.P. method (*17*). An accurately known solution containing 5 p.p.m. of nitrite ion was prepared by suitable dilution of an aliquot. This solution is stable when protected from bacteria and carbon dioxide of the air. The use of boiled water helps eliminate both bacteria and carbon dioxide. One milliliter of chloroform per liter can be added to prevent bacterial growth and does not interfere with the color reaction. One-tenth gram or less of sodium hydroxide per liter may be added to prevent the liberation of the unstable nitrous acid by carbon dioxide of the air. The solution should have a pH of 8 to 11.5.

For this investigation a solution of 4-aminobenzenesulfonic acid was prepared by dissolving 0.60 gram of recrystallized material in 100 ml. of distilled water. Heating aids dissolution. The 1-aminonaphthalene hydrochloride solution was prepared by dissolving 0.60 gram of recrystallized material in 100 ml. of distilled water. The 4-aminobenzenesulfonic acid and the 1-aminonaphthalene hydrochloride are colorless solids when pure, but, especially the latter, tend to discolor on exposure to light and air. Discolored reagents should be recrystallized. The 4-aminobenzenesulfonic acid can be recrystallized from hot water. To recrystallize from 5 to 10 grams of 1-aminonaphthalene hydrochloride, add 2 grams of decolorizing charcoal (Norite) and 100 ml. of water and boil for a few minutes. Rapidly filter the hot mixture through a Büchner funnel fitted with a filter paper. If the filtrate is not clear and colorless, add another 2 grams of decolorizing charcoal, boil again, and refilter. Then add 25 ml. of concentrated hydrochloric acid to the filtrate and cool to 0° C. in an ice bath. Filter off the recrystallized hydrochloride on a Büchner funnel, air-dry the product on a porous plate in a dark place, and store it in a tightly closed, dark bottle. Hydrochloric acid solutions of this reagent show much less tendency to discolor than acetic acid solutions which are used by some.

Color Reaction. The color formed by 1-aminonaphthalene with diazotized 4-aminobenzenesulfonic acid is a reddish purple which has its minimum transmittancy near 520 mμ (Figure 1). The reactions involved may be indicated by the following equations:



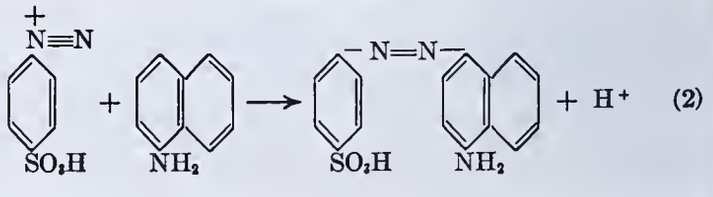


Table I. Effect of pH on Reaction Rates at 30° C.

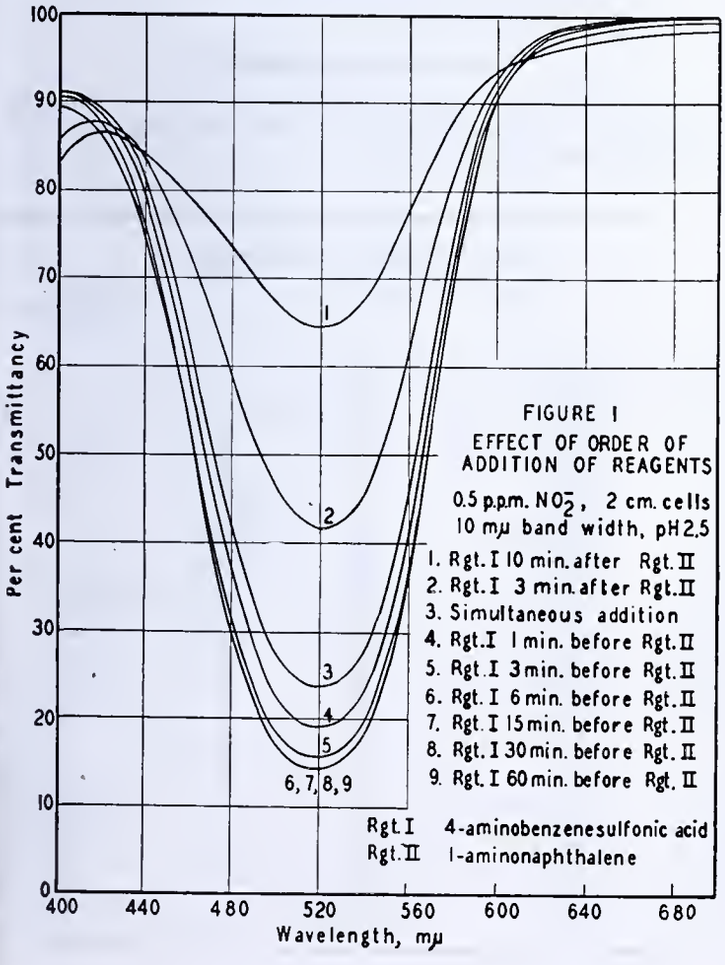
pH	Minutes Required: Diazotization	Coupling
1.12	1	60
1.38	..	30
1.64	..	20
2.00	3	10
2.15	..	7
2.29	..	5
2.58	6	3
3.00	6	2
3.38[a]	9	..
3.59[a]	12	..
3.67[a]	15	..

[a] Incomplete color development at these pH values.

EFFECT OF REAGENT CONCENTRATION. For 50 ml. of a solution containing 0.4 p.p.m. of nitrite, a minimum of 0.20 ml. of 4-aminobenzenesulfonic acid solution and 0.20 ml. of 1-aminonaphthalene solution is required. A tenfold excess of either reagent has no further effect upon the color developed. The acid required was added independently to maintain uniform acidity.

EFFECT OF ORDER OF ADDITION OF REAGENTS. The order of addition of reagents is of utmost importance. Figure 1 shows that the diazotization reaction with 4-aminobenzenesulfonic acid must be complete before 1-aminonaphthalene is added. Any unreacted nitrite still present when the coupling agent is added is partially destroyed by the amino group of the latter.

EFFECT OF pH. To establish the most desirable acidity, samples were buffered at different pH values by means of hydrochloric acid, hydrochloric acid–potassium chloride and acetic acid–sodium acetate combinations. The diazotization and coupling times required at room temperature for these various acidities are listed in Table I. These values were determined from curves similar to those in Figure 1. In Figure 1, 6 minutes' diazotization time is required at a pH of 2.5. It was further noted that full color is developed within 3 minutes after the addition of the coupling agent. High acidities increase the diazotization rate and decrease the coupling rate. Low acidities decrease



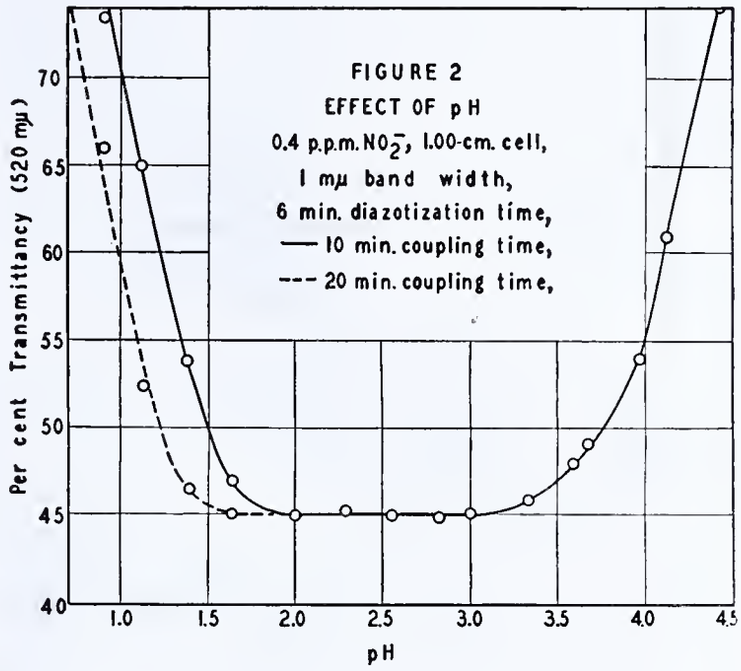


Table II. Effect of Temperature on Reaction Rates

Process	pH	Minutes Required: 20° C.	30° C.
Acetic Acid Method (*1*)			
Diazotization	3.1	12	9
Coupling	2.8	3	2
Hydrochloric Acid Method (*13*)			
Diazotization	2.1	9	6
Coupling	2.0	12	10
Hydrochloric Acid–Sodium Acetate			
Diazotization	1.4	2	1
Coupling	2.5	4	3

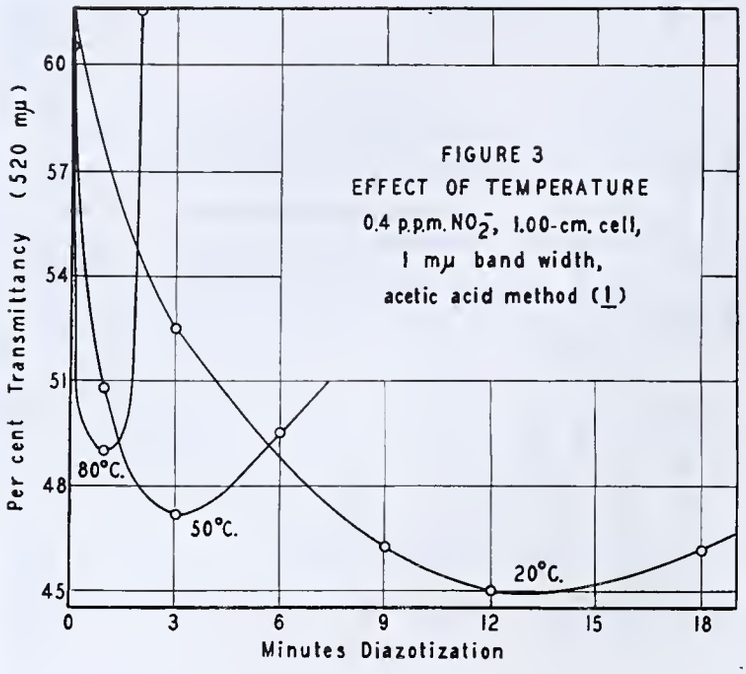
the diazotization rate and increase the coupling rate. This might have been predicted by the principle of mass action from Equations 1 and 2 in which the hydrogen ion is a reactant and a product, respectively.

The transmittancies at 520 mμ for these samples at various pH values are plotted in Figure 2, allowing 6 minutes' diazotization time and 10 minutes' coupling time, which were chosen as reasonable times. As indicated in Table I, incomplete color development in high acidities is due to incomplete coupling and in low acidities is due to incomplete diazotization. Increasing the coupling time brings about full color development in slightly more acidic samples, but increasing diazotization time does not bring about full color development in samples of lower acidity.

The colorimetric stability of the system is found to increase as the acidity increases.

After a study of Table I, it was decided to add 20 ml. of concentrated hydrochloric acid to the first reagent before diluting it to 100 ml. The resulting pH during diazotization is about 1.4 After adding the second reagent, the system is buffered with sodium acetate to a pH which will permit reasonably rapid coupling combined with a reasonable color stability. A pH of from 2.0 to 2.5 is considered best for general purposes. However, a slowly coupling, stable system can be had by buffering to a pH of 1.7 to 2.0, or a rapidly coupling, briefly stable system can be had by buffering to a pH of 2.5 to 3.0.

EFFECT OF TEMPERATURE. Figure 3 shows the effect of temperature on the diazotization reaction at a pH of 3.1. Since the reaction at this pH is slow, any effect of temperature can be readily measured. As the temperature increases, the rate of reaction increases as indicated in Table II and Figure 3. It is



evident, however, that the diazotization product is unstable both on heating and on long standing. In the highly acidic solution herein recommended, the reaction proceeds rapidly without heating, and the diazonium salt formed is relatively stable.

Effect of Light. This determination should be carried out in artificial light of moderate intensity. The reaction mixture should be shielded from direct sunlight, especially during diazotization, because, as Holbourn and Pattle (*7*) have found, diazotization products are photochemically unstable.

Effect of Nitrite Concentration. The lower limit of nitrite content which can be determined by this method is fixed by the maximum cell length available; the upper limit is fixed by the solubility of the colored dye. The exact concentration at which the dye precipitates is difficult to determine, as it varies under different conditions. Under the conditions here recommended, concentrations up to 0.6 p.p.m. of nitrite do not precipitate within 30 minutes. Beer's law holds up to a concentration of 0.6 p.p.m. for a 10 mμ band width. Reliable determinations can be made in the range of 0.025 to 0.600 p.p.m. of nitrite ion in a 2-cm. cell. As little as 0.0005 p.p.m. of nitrite nitrogen can be determined by the use of 24-cm. Nessler tubes.

Effect of Diverse Ions. In general, the effect of 400 p.p.m. of diverse ions on the analysis of a solution containing 0.4 p.p.m. of nitrite was determined. The quantities of interfering diverse ions which may be present without causing an error greater than 2% in the nitrite content are shown in Table III.

Of the diverse ions studied, the following did not interfere when present in concentrations 1000 times that of the nitrite: barium, beryllium, calcium, lead (II), lithium, magnesium, manganese (II), nickel (II), potassium, sodium, strontium, thorium, uranyl, zinc, arsenate, benzoate, borate, bromide, chloride, citrate, fluoride, formate, iodate, lactate, molybdate, nitrate, oxalate, phosphate, pyrophosphate, salicylate, selenate, sulfate, tartrate, tetraborate, and thiocyanate.

The interfering ions fall into various classes. Amines, oxidizing agents, and reducing agents destroy nitrites. Some ions complex the nitrite and retard its activity. Some ions precipitate under reaction conditions; others upset the acidity conditions; and still others interfere because of their own color.

Amines such as ammonia, urea, and aliphatic primary amines (HNH_2, NH_2CONH_2, and RNH_2) react with nitrites to liberate gaseous nitrogen. Small concentrations of ammonium ion did not interfere in this study but high concentrations should be avoided.

Nitrites are destroyed by reducing ions such as iodide, iron (II), chlorostannite, sulfide, thiosulfate, and sulfite, which must be absent from the sample. Strong oxidizing ions such as permanganate, chlorate, trisulfatocerate, perchlorate, periodate, peroxydisulfate, and tungstate should be absent. Other oxidizing ions such as dichromate, iodate, and selenate did not show any destruction of nitrite in these extreme dilutions in the time allowed in this study, but they may on longer standing.

Mercury (I) and silver precipitate as their chlorides, and bismuth and antimony (III) presumably as their oxychlorides in the presence of the hydrochloric acid used. Lead (II) also precipitates as its chloride in concentrated solution but redissolves at room temperature upon dilution to 400 p.p.m. and causes no interference. Chloroplatinate, iron (III), gold (III), and metavanadate ions form precipitates with 1-aminonaphthalene.

Alkali salts of ions such as carbonate, acetate, cyanide, and silicate reduce the acidity of the system and should be present only in limited quantities. The chlorostannate solution used contained hydrochloric acid. Its high acidity limited the amount to 40 p.p.m.

Mercury (II) causes high results, whereas copper (II) catalyzes the decomposition of the diazonium salt, causing low results. Both should be absent.

Purple ions, such as cobalt (II), which absorb green light, should be limited in concentration; whereas green ions, such as nickel (II) have little effect. Pale yellow ions, such as uranyl, show little interference; whereas more intensely yellow dichromate should be limited to 80 p.p.m. Chromium (III) should be limited to 40 p.p.m. All these colored ions change the hue of the system and must be absent for visual comparison.

DISCUSSION

In the present investigation, four requirements of a satisfactory method were found:

1. Diazotization should be carried out in strongly acidic solution.
2. Diazotization should be carried out in as cool a solution as is practicable.
3. Coupling should not be attempted until diazotization is complete.
4. Coupling should be carried out in as low an acidity as is consistent with colorimetric stability.

The acetic acid procedures (*1*, *8*) do not meet the first requirement; the elevated temperature procedures (*8*, *12*, *16*) do not meet the second; the mixed reagent procedures (*11*, *12*, *16*) do not meet the third; and the hydrochloric acid procedures (*13*) do not meet the fourth requirement.

The recommended procedure follows:

RECOMMENDED PROCEDURE

Sample. *Selection and Preparation.* Procure a representative portion of the material to be analyzed, and subject it to the necessary preparative treatment.

Table III. Effect of Diverse Ions

Ion	Added as:	Present *P.p.m.*	Error %	Amount Permissible *P.p.m.*
Au^{+++}	$AuCl_3$	5	Ppt.	0
Sb^{+++}	$SbCl_3$	400	Ppt.	0
Bi^{+++}	$Bi(NO_3)_3$	400	Ppt.	0
Ce^{++++}	$(NH_4)_2Ce(NO_3)_6$	20	New hue	0
Cr^{+++}	$Cr_2(SO_4)_3$	80	3	40
Co^{++}	$Co(NO_3)_2$	400	8	100
Cu^{++}	$CuSO_4$	20	7	0
Fe^{++}	$FeSO_4$	40	4	0
Fe^{+++}	$FeCl_3$	10	Ppt.	0
Hg^{++}	$Hg(NO_3)_2$	400	50	0
Hg_2^{++}	$Hg_2(NO_3)_2$	400	Ppt.	0
Ag^+	$AgNO_3$	400	Ppt.	0
$C_2H_3O_2^-$	$NaC_2H_3O_2$	400	3	200
CO_3^{--}	Na_2CO_3	200	2	200
ClO_3^-	$KClO_3$	40	4	0
$PtCl_6^{--}$	H_2PtCl_6	80	2	80
$SnCl_6^{--}$	H_2SnCl_6	80	3	40
$SnCl_4^{--}$	H_2SnCl_4	40	90	0
CN^-	KCN	200	4	100
$Cr_2O_7^{--}$	$K_2Cr_2O_7$	80	2	80
I^-	KI	10	3	0
ClO_4^-	$KClO_4$	400	15	0
IO_4^-	KIO_4	20	3	0
MnO_4^-	$KMnO_4$	1	14	0
$S_2O_8^{--}$	$(NH_4)_2S_2O_8$	400	37	0
SiO_3^{--}	Na_2SiO_3	400	5	200
SO_3^{--}	Na_2SO_3	40	88	0
S^{--}	Na_2S	10	80	0
$S_2O_3^{--}$	$Na_2S_2O_3$	40	23	0
WO_4^{--}	Na_2WO_4	40	3	10
VO_3^-	KVO_3	20	Ppt.	0

Measurement. Weigh or measure by volume a quantity of sample containing 0.03 mg. or less of nitrite.

Treatment. Most samples in water analysis need no further treatment. The proper treatment of other materials depends on the nature of the sample. Basic or acidic samples are neutralized with hydrochloric acid or sodium hydroxide.

DESIRED CONSTITUENT. *Separation.* If interfering ions are present, they should be complexed or removed to within the permissible concentrations given in Table III.

Measurement. To the sample in a 50-ml. volumetric flask add 1.0 ml. of acidified 4-aminobenzenesulfonic acid reagent. (To prepare this reagent, completely dissolve 0.60 gram of 4-aminobenzenesulfonic acid in about 70 ml. of hot water, cool the solution, add 20 ml. of concentrated hydrochloric acid, dilute to 100 ml. with water, and mix thoroughly.) Mix well. Allow at least 3, and not more than 10, minutes for diazotization at room temperature in diffuse light. Then add 1.0 ml. of 1-aminonaphthalene hydrochloride reagent (0.60 gram of 1-aminonaphthalene hydrochloride and 1.0 ml. of concentrated hydrochloric acid diluted to 100 ml. with water) and buffer the system to a pH of 2.0 to 2.5 with sodium acetate. This requires about 1.0 ml. of filtered 2.0 *M* sodium acetate solution. Dilute to volume and mix well. After 10 minutes, measure the intensity of the reddish purple color by the usual means. All measurements should be made within 30 minutes. Spectrophotometric measurements can be made at 520 mμ. A green filter, such as a Corning No. 401 of suitable thickness, is recommended for filter photometers. Permanent standards for visual comparisons have been suggested (*4*).

For minute amounts of nitrite, add the reagents according to the above procedure to 50 ml. of sample in a Nessler tube. The color developed is compared with a series of temporary or permanent standards.

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ABSTRACTED from a thesis presented by B. F. Rider to the Graduate School of Purdue University in partial fulfillment of the requirements for the degree of master of science, October, 1944.

Rapid Estimation of Effect of Pressure upon Boiling Points of Organic Compounds

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IT IS frequently convenient in laboratory distillations under reduced pressure to have at hand a means for estimating rapidly the boiling point of a substance at pressures other than that being used. A special case is finding the approximate boiling point at atmospheric pressure by extrapolation from a single boiling point value obtained at some reduced pressure. Another useful application is in selecting in advance a suitable reduced pressure for distilling a compound whose boiling point at atmospheric pressure is known. In these cases, a high degree of accuracy is usually unnecessary, and the consideration of accuracy is offset by the convenience of having a generally applicable process of calculation or extrapolation.

Numerous methods have been proposed for estimating boiling points at reduced pressure or finding the vapor pressure of substances at various temperatures. One of the most useful is that of Cragoe (*1*) as modified by Hass and Newton (*4*), which employs the equation:

$$\Delta t = t' - t = \frac{(273.1 + t)\ (2.8808 - \log p)}{\phi + 0.15\ (2.8808 - \log p)} \qquad (1)$$

where t' = boiling point, ° C. at atmospheric pressure
t = boiling point, ° C. at pressure p (mm.)
ϕ = entropy of vaporization at 760 mm. divided by 2.3 R

Hass and Newton have modified Cragoe's classification of compounds into eight groups according to their physical or structural relationship, and have established values for ϕ for each group.

However, calculation using this equation is rather cumbersome and time-consuming. The object of the present paper is to reformulate the Hass-Newton equation, and to derive graphs suitable for rapid interconversion and for use especially in the ordinary laboratory type of distillation.

Equation 1 may be rearranged to give:

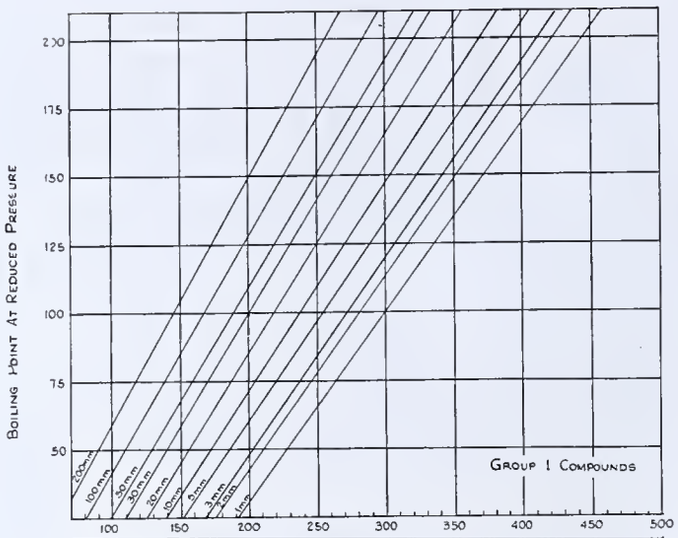
$$t = t' \frac{(\phi + 0.15\ P)}{(\phi + 1.15\ P)} - \frac{273.1\ P}{\phi + 1.15\ P} \qquad (2)$$

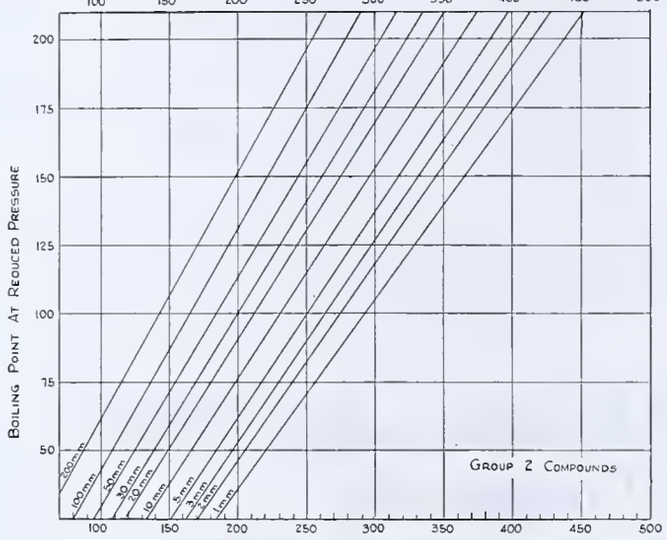
where $P = 2.8808 - \log p$.

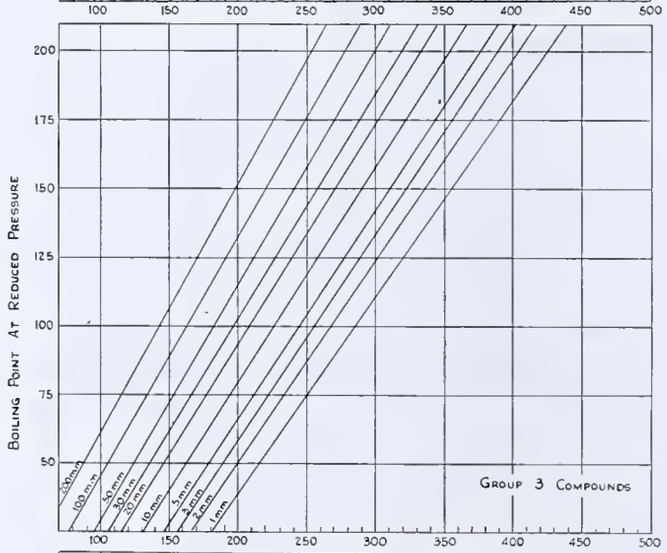
By the use of this equation and the entropy values given by Hass and Newton (*3*), graphs showing the relationship between

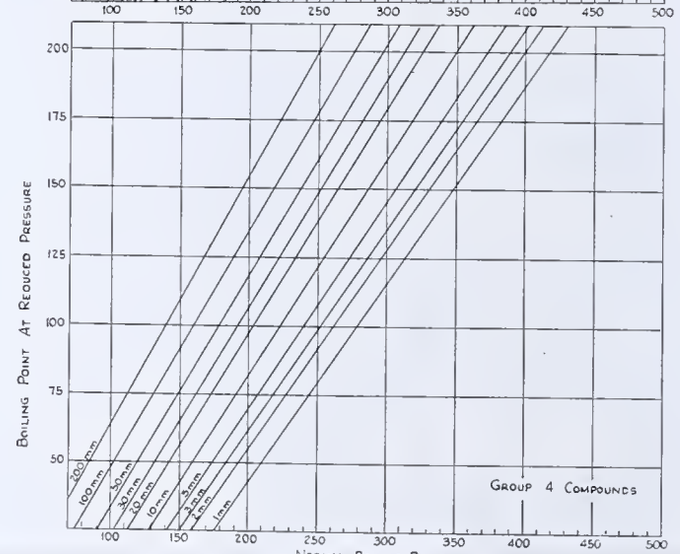
Table I. Classification of Compounds According to Groups

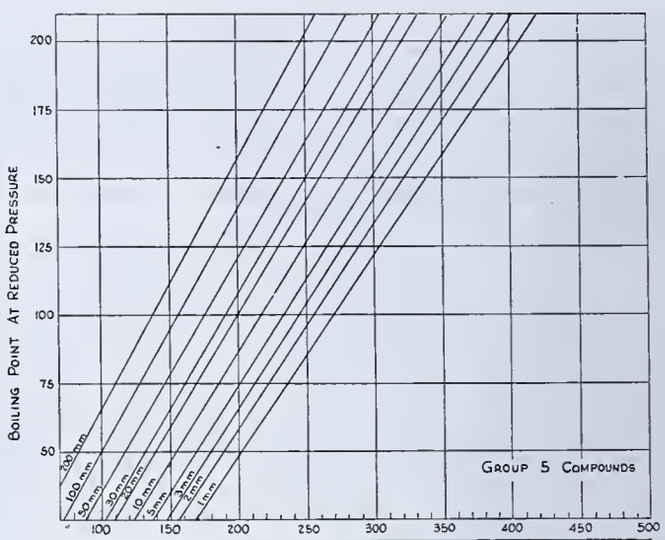
Compound	Group	Compound	Group
Acetals	3	Heptylic acid	7
Acetic acid	4	Hydrocarbons, sat.	2
Acetic anhydride	6	Hydrocarbons, unsat.	1
Acetophenone	4	Isoamyl alcohol	7
Amines	3	Isobutyl alcohol	8
n-Amyl alcohol	8	Isobutyric acid	6
Anthracene	1	Isocaproic acid	7
Anthraquinone	1	Lauric acid	5
Benzaldehyde	2	Laurylamine	7
Benzene	2	Methyl salicylate	2
Benzoic acid	5	Myristic acid	5
Benzonitrile	2	Naphthalene	2
Benzophenone	2	α- and β-Naphthols	3
Benzoyl chloride	3	Nitroalkanes	3
Bromobenzene	2	Nitrobenzene	3
Butyric acid	7	Nitrotoluenes	2
Camphor	2	Nitrotoluidines	2
Caprylic acid	5	Allo-ocimene	1
Chloroanilines	3	Octanols	8
Chlorobenzene	2	Oleic acid	5
Cresols	4	Phenanthrene	1
Coumarin	2	Phenol	5
Dibenzyl ketone	2	Phthalic anhydride	2
Dimethyl oxalate	4	α- and β-Pinenes	1
Esters	3	Propionic acid	5
Ethers	2	*n*-Propyl alcohol	8
Ethylene glycol	7	Quinoline	2
Ethylene oxide	3	Sebacic acid	7
Formic acid	3	Stearic acid	5
Furfural	2	Sulfides	2
Glycols	7	Valeric acid	7
Glycol diacetate	4	Water	6
Halogen derivatives	Same group as where $X = H$		

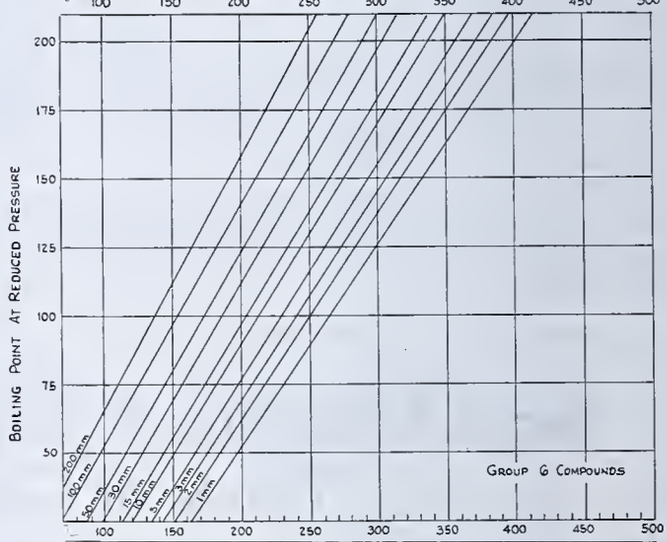

Boiling Point At Reduced Pressure
Group 1 Compounds
200 mm
100 mm
50 mm
30 mm
20 mm
10 mm
5 mm
3 mm
2 mm
1 mm

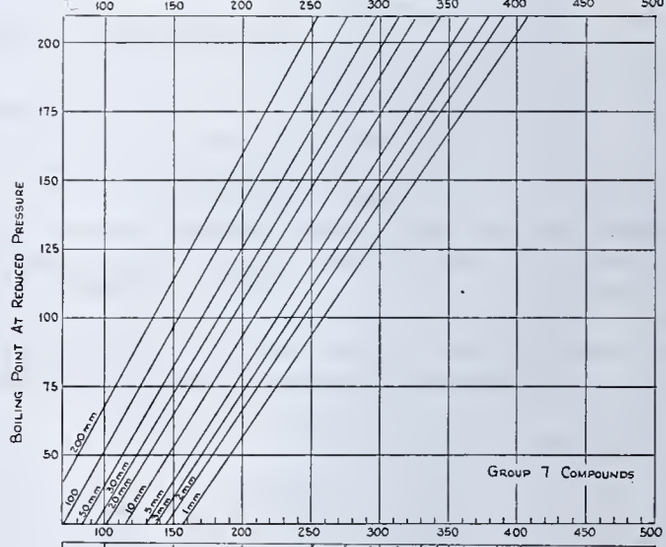

Boiling Point At Reduced Pressure
Group 2 Compounds
200 mm
100 mm
50 mm
30 mm
20 mm
10 mm
5 mm
3 mm
2 mm
1 mm


Boiling Point At Reduced Pressure
Group 3 Compounds
200 mm
100 mm
50 mm
30 mm
20 mm
10 mm
5 mm
3 mm
2 mm
1 mm


Boiling Point At Reduced Pressure
Group 4 Compounds
200 mm
100 mm
50 mm
30 mm
20 mm
10 mm
5 mm
3 mm
2 mm
1 mm
Normal Boiling Point


Boiling Point At Reduced Pressure
Group 5 Compounds
200 mm
100 mm
50 mm
30 mm
20 mm
10 mm
5 mm
3 mm
2 mm
1 mm


Boiling Point At Reduced Pressure
Group 6 Compounds
200 mm
100 mm
50 mm
30 mm
15 mm
10 mm
5 mm
3 mm
2 mm
1 mm


Boiling Point At Reduced Pressure
Group 7 Compounds
200 mm
100
50 mm
30 mm
20 mm
10 mm
5 mm
3 mm
2 mm
1 mm

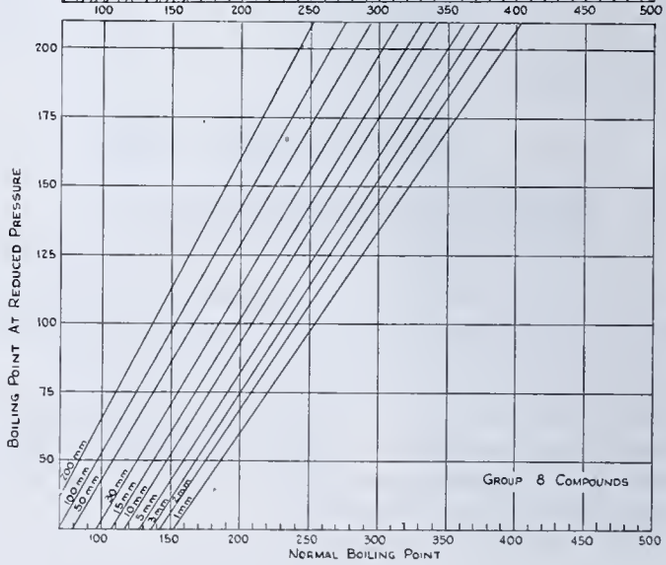

Boiling Point At Reduced Pressure
Group 8 Compounds
200 mm
100 mm
50 mm
30 mm
15 mm
10 mm
5 mm
3 mm
2 mm
1 mm
Normal Boiling Point

Table II. Comparison of Observed and Estimated Boiling Points

Compound (Group) Boiling point	Coumarin (2) Obs.	Coumarin (2) Est.	Acetophenone (4) Obs.	Acetophenone (4) Est.	Ethylene Glycol (7) Obs.	Ethylene Glycol (7) Est.
Pressure						
200 mm.	...	...	154	155	158	158
100 mm.	220	218	133	135	140	139
50 mm.	196	196	115	118	125	124
20 mm.	171	170	94	96	105	105
10 mm.	154	152	81	81	92	91
5 mm.	139	136	69	70	80	79

the normal boiling point and the boiling point at reduced pressure for the eight groups of compounds have been constructed. The classification of the compounds and families of compounds given in Table I is adapted from the reference articles (*3*, *4*). It is suggested that compounds not given in the table be classified in the group with compounds which they most closely resemble. In this connection, Cragoe (*1*) has pointed out that higher members of a series of compounds are usually in the same group, while the first members are generally in a different group. Boiling point values for intermediate pressures may be obtained from the charts by interpolation.

In general, the observed and estimated values have been found to agree satisfactorily. Representative results are shown in Table II, which gives a comparison of estimated and observed values for typical compounds of different groups. The observed values given for ethylene glycol have been obtained by interpolation of values reported by de Forcrand (*2*).

ACKNOWLEDGMENT

The author wishes to thank H. B. Hass and R. F. Newton for their valuable comments.

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Nephelometric Determination of Small Amounts of Sodium

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A rapid nephelometric method for determining small amounts of sodium salts in either liquids or solids is disclosed. An alcoholic uranyl magnesium acetate reagent is employed, and comparative data are given to indicate the sensitivity of various alcoholic compositions. The method is accurate to approximately 1 grain per gallon of sodium salts, expressed as sodium chloride in water analysis, and to ±0.003%, expressed as sodium oxide on solid samples. The method is particularly adaptable to routine analytical problems.

THE advantages of employing alcoholic magnesium uranyl acetate reagents, or of incorporating alcohol in some manner before the precipitation of the sodium uranyl acetate triple salt, have been disclosed by several workers (*1*, *2*, *3*). The need for a very rapid, sensitive method for determining traces of sodium salts in a solid product suggested the advisability of trying to employ an alcoholic reagent in the development of a nephelometric procedure. By modifying the method of Caley, Brown, and Price (*1*), a satisfactory procedure has been developed which is particularly useful for control analyses in the manufacture of solids with low sodium salt impurity specifications, but which may also be adapted to rapid estimation of sodium salt concentrations in liquids.

APPARATUS

The photometer used in this investigation employed a Nalco blue photofilter, a General Electric light-sensitive cell (Catalog No. 88 × 565), a General Electric Mazda lamp No. 51 (6 to 8 volts), and an ammeter (Model 26) manufactured by Simpson Electric Co., Chicago, Ill.

The specifications on the construction of the apparatus are:

Distance from light source to cell	50 mm.
Distance from light source to filter	25 mm.
Slit in screen	6.5 mm. wide × 16 mm. high
Length of path of light through solutions	16 mm.
Thickness of cell walls	2 mm.

REAGENT AND SOLUTIONS

ALCOHOLIC MAGNESIUM URANYL ACETATE. To 30 grams of uranyl acetate dihydrate, 150 grams of magnesium acetate tetrahydrate, and 20 ml. of glacial acetic acid are added 500 ml. of alcohol and sufficient water to make up to 1 liter. The resultant is heated on the steam bath, with stirring, until the salts are dissolved. Care must be taken to lose as little solvent as possible during the solution step. The reagent is then stirred until cool, and filtered without further dilution into a brown glass bottle.

Potassium chloride, lithium chloride, and sodium chloride solutions were prepared by dissolving the pure chemicals in triply distilled water.

PRELIMINARY EXPERIMENTS

Although, after an extensive investigation on the value of precipitating the triple salt in an alcoholic medium, Greene (*2*) came to the conclusion that the alcohol could not be incorporated into the reagent, the ease of controlling the precipitation medium when using only one reagent was especially appealing for the development of a nephelometric procedure where uniform crystal growth is imperative. In their earlier work, Caley, Brown, and Price (*1*) had successfuly employed an alcoholic reagent, and the authors' preliminary work was done using a slight modification of their reagent, but substituting the nephelometric procedure for their more complex and critical centrifugal estimation method. The study of reagents prepared using various alcohols was the initial step in the investigation of the method. In Figure 1 are plotted representative transmittancy curves for reagents made from methanol, ethanol, isopropanol, a mixture of ethanol and

Table I. Comparison of Sensitivity of Reagent Prepared from Methanol-Ethanol Mixtures

NaCl Added	Ethanol	9 Volumes Ethanol, 1 Volume Methanol	8 Volumes Ethanol, 2 Volumes Methanol	7 Volumes Ethanol, 3 Volumes Methanol	6 Volumes Ethanol, 4 Volumes Methanol	Methanol
Grains/gal.	*Per cent transmittancy*					
0	91.9	91.0	91.5	91.0	90.0	90.0
2	88.0	87.0	86.5	88.0	88.5	90.0
5	83.0	83.0	82.5	83.0	84.0	90.0
10	77.0	78.0	77.0	77.0	80.0	88.0
15	72.0	73.0	73.0	73.0	76.5	84.0
20	69.5	69.5	70.0	69.5	71.0	80.0
25	67.0	67.5	67.5	67.0	69.5	75.0

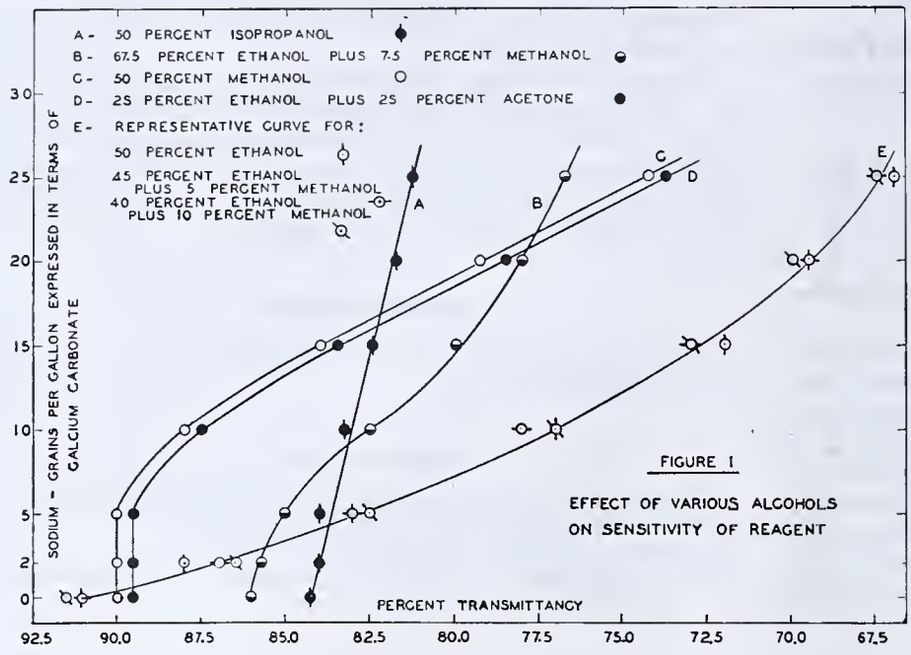

FIGURE 1
EFFECT OF VARIOUS ALCOHOLS ON SENSITIVITY OF REAGENT

Table II. Stability and Reproducibility of Reagent

NaCl Added	Reagent Batch 1	Reagent Batch 2	Reagent Batch 3
Grains/gal.		*Per cent transmittancy*	
0	91.0	91.5	90.5
2	87.0	87.0	86.5
5	83.5	83.0	83.0
10	79.0	78.0	78.0
15	73.0	74.0	73.0
20	69.5	69.5	69.5
25	67.5	68.0	68.0
35	63.0	63.0	63.0

Table III. Analysis of Solutions

	Water A	Water B
Total hardness as $CaCO_3$, grains per gallon	8.7	37.0
Calcium as $CaCO_3$, grains per gallon	5.2	30.0
Sodium as NaCl, grains per gallon	1.5	7.9
Silica, p.p.m. SiO_2	9.7	10.7

NaCl Added	NaCl Found	NaCl Added	NaCl Found
	Grains per gallon		
0.0	1.5	0.0	7.9
5.0	6.5	5.0	12.9
10.0	11.5	10.0	17.9
17.5	19.0	17.5	23.5

acetone, and a representative curve for methanol-ethanol mixtures.

From these data, it is apparent that ethanol reagents, or ethanol reagents containing small quantities of methanol, are more sensitive than methanol or isopropanol reagents. Incorporating acetone, or increasing the alcohol content above 50% by volume, rendered the reagent less sensitive. The disadvantage of an increase in the alcohol content of the precipitation medium was apparently due to the enhanced crystal growth, which caused the precipitated sodium salt to settle very rapidly, thereby decreasing the accuracy of the transmittancy measurement. The possibility of using ethanol denatured with methanol was of special interest because of the relative ease of obtaining such mixtures. Table I shows the effect of increased methanol-ethanol ratios. Volume ratios of 3 to 7 can be tolerated before the detrimental influence of methanol is appreciable. Therefore, because of the availability of Formula 30 alcohol (10% methanol and 86% ethanol by volume), it was selected for the preparation of the reagent. Table II gives data illustrating the stability and reproducibility of this alcoholic reagent. Reagents 1, 2, and 3 were prepared about 5 days apart. The results are well within the limits of accuracy of the method.

PROCEDURE

To 2 ml. of the solution to be tested in the test cell are added one drop of C.P. concentrated hydrochloric acid and 15 ml. of the alcoholic reagent. The two solutions are mixed by inverting five times, allowed to stand 5 minutes, and again mixed by inverting five times. After an additional 5-minute period of standing, the transmittancy is read and the sodium content determined by interpolation from a previously prepared standard transmittancy curve.

RESULTS

SOLUTIONS. In Table III are tabulated the results obtained by adding known amounts of sodium ion as sodium chloride to natural raw waters. The accuracy of this rapid procedure is approximately 0.000015 gram per cc. (1 grain per gallon) expressed in terms of sodium chloride.

Relatively high concentrations of lithium and potassium ions do not affect the accuracy of the sodium determination. The results obtained, using a standard 10 grains per gallon sodium chloride water to which was added varying amounts of lithium and potassium salts, are shown in Table IV.

SOLIDS. The procedure was developed primarily for analytical control in the manufacture of fluid cracking catalyst for the petroleum industry. It was found that nearly 100% of the sodium salts could be extracted from this siliceous material by boiling for 2 minutes in 1 to 1 hydrochloric acid.

To 10 grams of catalyst are added 20 ml. of 1 to 1 hydrochloric acid, and the mixture is boiled exactly 2 minutes. After filtering, 10 ml. of alcoholic reagent are added to 2 ml. of filtrate, the two solutions mixed by inverting 5 times, allowed to stand 5 minutes, and mixed again by inverting 5 times, and the transmittancy is determined after an additional 5 minutes. In Table V, the results of this rapid method are compared with results obtained by the standard gravimetric procedure. The accuracy of the method can be considered to be 0.003% expressed as Na_2O.

Table IV. Interference of Potassium and Lithium

NaCl Present	Salt Added	% Transmittancy
Grains/gal.	*Grains/gal.*	
10	None	88.0
10	LiCl 2.5	88.0
10	LiCl 5.0	88.0
10	LiCl 10.0	87.5
10	LiCl 20.0	88.0
10	KCl 2.5	87.5
10	KCl 5.0	88.0
10	KCl 10.0	88.0
10	KCl 20.0	88.0

Table V. Analysis of Solid Materials

Sample	Na_2O Gravimetric %	Na_2O Found %
1	0.0195	0.017
2	0.010	0.008
3	0.019	0.021
4	0.012	0.009

LITERATURE CITED

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Colorimetric Determination of Phenols

Application to Petroleum and Allied Products

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A colorimetric method is described for determination of the total phenolic content of hydrocarbon mixtures, organic solvents, aqueous solutions, and concentrated phenols. The method is not affected by organic materials containing other functional groups (such as aldehydes or ethers) nor by water, mineral acids, inorganic bases, or inorganic salts. In this procedure the sample, or a caustic extract thereof, is dissolved in acetic acid, nitrous acid solution is added to form the respective nitrosophenol, and after a short reaction period an excess of alcoholic ammonium hydroxide is added. After standing, the intensity of the resulting colored quinoid salt is measured with a photoelectric colorimeter and related to that of a calibration curve which has been prepared under identical conditions by concentrations of a standard phenol sample. Although the method was primarily developed for the determination of monohydroxy alkyl phenols in refined petroleum products, it has been successfully applied to the determination of monohydroxy- and dihydroxyphenols in a variety of materials such as crude oil, oil additives, solvents, polymers, plastics, cresylic acids, and caustic treating solutions.

DURING the past decade a need has developed for the determination of phenols, particularly monohydroxyphenols, in a variety of materials. This need has become increasingly important to the petroleum chemist because the presence of phenols often affects the behavior of many petroleum products. For example, the presence of small amounts of phenols in paint and varnish thinners has been known to retard the drying rate of these products, and phenolic compounds have found use in gasoline as antioxidants to prevent gum formation, light instability, etc. Experience has shown that there is need of a general method because the phenolic compounds encountered are usually a mixture of various unknown phenols and because it is necessary to apply the method in the presence of materials such as alcohols, acids, amines, ethers, aldehydes, ketones, water, organic sulfur derivatives, and inorganic compounds. Such characteristics as rapidity and accuracy are secondary in importance to precision and universal applicability.

A review of the literature reveals a number of qualitative tests for phenols, most of which have been studied and classified by Gibbs (*2*). Only a few of the qualitative tests are adaptable to the quantitative determination of phenols because many of the reactions are applicable only to certain types of phenols and because many are not specific for phenols, reacting similarly with other types of compounds.

Included among these methods of limited application are those of Houghton and Pelly (*4*), which involves oxidation of the phenol to an indophenol by hypochlorite in the presence of *p*-aminodimethylaniline, of Gibbs (*3*), in which the phenol is reacted with quinone chloroimide to form a colored indophenol, and of Folin and Denis (*1*), wherein phenolic compounds of phosphomolybdicphosphotungstic acid are reduced to the lower oxides of molybdenum and tungsten, resulting in the formation of blue solutions.

Stoughton (*5*) devised a method which depends upon treatment of the phenol with nitric and sulfuric acids at approximately 100° C. to form a nitrosophenol which rearranges in the presence of excess alcoholic ammonium hydroxide to form the highly colored quinoid radical. The probable reactions are:

$$2HONO_2 \xrightarrow{\text{organic matter}} 2HONO + O_2$$

$$RC_6H_4OH + HONO \xrightarrow{H_2SO_4} RC_6H_3\begin{matrix}OH\\NO\end{matrix} + H_2O$$

$$RC_6H_3\begin{matrix}OH\\NO\end{matrix} \xrightarrow{NH_4OH} \left[RC_6H_3\begin{matrix}O\\NO\end{matrix}\right]^- + H^+$$

The resulting colored solutions are compared with those from standard phenols by means of a visual colorimeter. This method is generally applicable to ortho, meta, and para substituted phenols. The colors produced by the reaction with different phenols range from greenish yellow to orange yellow. Wetlaufer, Van Natta, and Quattlebaum (*6*) used a modified version of the Stoughton procedure, applying it to the determination of traces of phenols in hydrocarbon solvents. They achieved high sensitivity by extracting the phenols with a small portion of dilute caustic solution.

The method presented here is a further modification of Stoughton's (*5*) nitrosophenol procedure. The significant differences are the use of sodium nitrite solution in place of nitric acid as the source of nitrous acid, formation of the nitrosophenol at room temperature under carefully predetermined conditions, and measurement of the colored solutions in a photoelectric colorimeter. As in the method of Wetlaufer *et al.* (*6*), the phenols are extracted from hydrocarbons prior to the color development. This procedure achieves greater sensitivity and avoids interference of large quantities of hydrocarbons. As a result of these changes, the proposed method is versatile, accurate, and applicable to a wide variety of materials.

APPARATUS

A sensitive photoelectric colorimeter equipped with light-absorption cells permitting passage of light through a depth of approximately 1 cm. of solution and equipped with a monochromatic light source consisting of either a lamp with color filters or a monochromator capable of producing light of narrow spectral range (50 mμ) which is predominantly violet (420 mμ).

REAGENTS

Potassium Hydroxide Solution, 10% aqueous.

Acetic Acid, C.P., glacial.

Buffer Solution. To prepare 1 liter of solution, mix 800 ml. of glacial acetic acid, 150 ml. of 10% potassium hydroxide solution, and 50 ml. of water.

Saturated Sodium Nitrite Solution, aqueous.

Alcoholic Ammonium Hydroxide Solution. To prepare 1 liter of solution, mix 450 ml. of anhydrous ethyl alcohol or isopropyl alcohol, 300 ml. of 14 *N* ammonium hydroxide, and 250 ml. of water.

Standard Phenol Solution, containing 0.1 mg. of the phenol to be determined per milliliter. Weigh accurately 0.1 ± 0.01 gram of the phenol in a 100-ml. volumetric flask and dilute to exactly 100 ml. with the buffer solution. Mix thoroughly, transfer a 10-ml. aliquot to a second 100-ml. volumetric flask, and dilute to the mark with the acetic acid buffer solution. Where maximum accuracy is essential be sure that the phenol used as a standard is the same as that determined. Where high accuracy is not essential, an arbitrary standard such as *p*-cresol may be used for the preparation of a calibration curve.

Sulfuric Acid, 36 *N*.

Diluent. Extract 1 liter of technical octane with 100 ml. of 10% potassium hydroxide solution and remove the aqueous layer. Extract a second time with the fresh caustic solution, then wash with 100 ml. of water and filter the hydrocarbon layer through a dry folded paper. When the sample is not soluble in octane, use other solvents such as benzene, toluene, ether, etc., purified in the same manner.

Table I. Relative Color Intensities Produced by Pure Phenols and Concentrated Phenol Mixtures

Material	Intensity[a] of Color Produced by 1 Mg. of Material Relative to Color from 1 Mg. of Petroleum Cresylic Acid (b.r. 220–225° C.)[b]
Petroleum cresylic acids	
Boiling range 220–225° C.[b]	1.00
Boiling range 200–220° C.[b]	1.42
Boiling range 200–225° C.[b]	1.34
Boiling range 200–235° C.[b]	1.06
Boiling range 220–240° C.[b]	0.81
Boiling range 225–245° C.[b]	0.79
Boiling range 235–290° C.[b]	0.56
Phenol	1.07
p-Cresol	1.24
o-Cresol	1.85
m-Cresol	0.73
p-Ethylphenol	1.21
o-Ethylphenol	1.82
m-Ethylphenol	0.76
2,4-Dimethylphenol	1.28
2,5-Dimethylphenol	0.68
2,6-Dimethylphenol	3.34
3,4-Dimethylphenol	0.93
3,5-Dimethylphenol	0.47
2,3,5-Trimethylphenol	0.52
2,4,6-Trimethylphenol	0.25
4-*tert*-Butyl-2,6-dimethylphenol	0.53
2,4-Dimethyl-6-*tert*-hutylphenol	0.22[c]
p-*tert*-Amylphenol	0.95
α-Naphthol	2.26
β-Naphthol	0.83
Resorcinol	0.98
o-Chlorophenol	0.80
m-Aminophenol	0.5
Guaiacol	2.02
8-Hydroxyquinoline	1.50
Hydroquinone	0.55
Catechol	0.55
p-*tert*-Butylcatechol	0.19[c]
Carvacrol	1.33
p-Hydroxybenzoic Acid	0.03

[a] Spekker photoelectric absorptiometer, No. 7 (violet) filter.
[b] Nominal boiling range, initial to 95% point.
[c] Measurement by Klett-Summerson photoelectric colorimeter, No. 42 (violet) filter.

PROCEDURE

Two procedures are described below. The choice of the procedure to be used depends upon the type of material which is being analyzed for phenol content. The direct procedure is generally used for oxygenated materials, while the extraction procedure is used for hydrocarbons.

Direct Procedure. Use this procedure for materials soluble in the acetic acid buffer solution—i.e., cresylic acids, alcohols, naphthenic acids, glycerols, phenyl ethers, ketones, and aqueous solutions. In the case of highly colored or opaque materials giving rise to high blanks, use the extraction procedure.

Into a 100-ml. volumetric flask introduce an accurately weighed sample of the size indicated below:

Phenol Content, %	Sample Size, Grams
0.0 to 0.4	2
0.3 to 0.8	1
0.6 to 1.6	0.5
1.5 to 4	0.2
3 to 8	0.1

For phenol contents above 8% use approximately smaller samples or preferably 0.5- to 5-ml. aliquots of solution of sample in buffer solution; in all such cases regulate sample or aliquot to contain 3 to 8 mg. of phenol. Dilute the sample to exactly 100 ml. with the buffer solution, mix, and transfer a 5-ml. aliquot into a 50-ml. volumetric flask. By means of a medicine dropper, add 5 drops of 36 *N* sulfuric acid and 2 drops of saturated sodium nitrite solution. Mix by swirling, allow the mixture to stand 15 to 30 minutes (30 to 45 minutes if hydroxybenzene is the phenol present), and then slowly add alcoholic ammonium hydroxide solution while cooling the flask in ice water to make a total volume of exactly 50 ml. at room temperature. Allow the final solution to stand 1 hour or preferably overnight and obtain a colorimeter reading using a violet light source (420 mμ).

Make three blank determinations according to the above procedure. In the first omit the sodium nitrite solution but include a duplicate aliquot of the sample solution; in the second omit the sample but include the nitrite solution; and in the third omit both the sample and the nitrite solution. For the most accurate work determine all three blanks for each analysis. Otherwise, determine all blanks at least once whenever new reagents are used; determine the first blank only when the sample is colored.

Extraction Procedure. Use this procedure for materials which are insoluble in the acetic acid buffer solution but soluble in the diluent or similar solvents. Always remove phenol contaminants from solvent by the treatment outlined upon preparation of the diluent.

Introduce 10 ml. of 10% potassium hydroxide solution into a 125-ml. Squibb-type separatory funnel and add the volumes of sample and diluent as indicated in the following tabulation:

Phenol Content *Mg./100 ml.*	Volume of Sample *Ml.*	Volume of Diluent *Ml.*
0 to 100	50 ± 0.2	0
50 to 200	25 ± 0.1	25
100 to 500	10 ± 0.1	40
200 to 1000	5 ± 0.05	45

For phenol contents greater than 1000 mg. per 100 ml., weigh a proportionately smaller quantity of sample and add 45 to 50 ml. of diluent.

Stopper the separatory funnel and shake for 5 minutes. Allow the mixture to stand in the separatory funnel until two distinct layers have formed and carefully remove the lower layer without loss into a 100-ml. volumetric flask. Add a 5-ml. portion of potassium hydroxide solution to the separatory funnel, shake the mixture for 5 minutes, allow the phases to separate, and quantitatively withdraw the lower layer into the same volumetric flask. Add 5 ml. of distilled water to the separatory funnel, shake for 2 minutes, allow the phases to separate, and withdraw the aqueous layer into the volumetric flask containing the caustic extracts.

To the combined extracts, slowly add glacial acetic acid while cooling the flask in ice water to make a total volume of exactly 100 ml. at room temperature. Pipet a 1- to 5-ml. aliquot containing 0.05 to 0.5 mg. of phenol into a dry 50-ml. volumetric flask. If necessary, add sufficient of the acetic acid buffer solution to make a total volume of 5 ml. Proceed with the color development as outlined under the direct procedure (above), starting with "by means of a medicine dropper add 5 drops of 36 *N* sulfuric acid, etc."

PREPARATION OF CALIBRATION CURVES

Prepare a calibration curve showing the relation between the colorimeter readings and the phenol content as follows:

From a buret or pipet introduce 0, 1, 2, 3, 4, and 5 ml. of the standard phenol solution into separate dry 50-ml. volumetric flasks. Add sufficient acetic acid buffer solution to make a total volume of 5 ml. and proceed with the color development as outlined under the direct procedure (above), beginning with "by means of a medicine dropper add 5 drops of 36 *N* sulfuric acid, etc."

CALCULATIONS

Correct the colorimeter reading obtained for the sample by means of the following equation:

$$\text{Corrected reading} = R - (B_1 + B_2 - B_3)$$

where

R = colorimeter reading for the sample
B_1 = colorimeter reading obtained when sodium nitrite solution is omitted
B_2 = colorimeter reading when sample is omitted
B_3 = colorimeter reading when both sample and sodium nitrite solution are omitted

Note. Unless the samples or extracts are colored, B_2 will be the only significant correction and B_1 and B_3 will cancel each other. Besides making a color blank, the degree of color interference may sometimes be minimized by reducing sample or aliquot size.

Convert the corrected colorimeter reading to milligrams of phenol by means of the prepared calibration curve and express the results as percentage by weight of phenol or as milligrams of phenol per 100 ml. Report the phenol used as standard.

EXPERIMENTAL

Apparatus Used. A Spekker absorptiometer (photoelectric) was used for the color measurements during the early part of the investigation. Later a Klett-Summerson photoelectric colorimeter and Fisher electrophotometer were used for the investigational work as well as actual application of the method. A Coleman spectrophotometer (Model 10S) was used to obtain the spectral transmittance curves of the colored solutions.

Relative Color Intensities Produced by Various Phenols. Samples containing exactly 1 mg. of a number of pure phenols and commercial cresylic acids were treated by the proposed direct procedure, and the intensities of the colors produced were measured photoelectrically with a Spekker absorptiometer using the No. 7 (violet) filter supplied with the apparatus. The materials tested and the data obtained are given in Table I, expressing the intensity of color produced by 1 mg. of material relative to that from 1 mg. of petroleum cresylic acid (nominal boiling range 220° to 225° C., initial to 95% point) taken as unity. The order of magnitude of color intensity values obtained by use of other photoelectric colorimeters or visual colorimeters agrees with those in Table I, but there are small differences depending upon the spectral characteristics of the light filter used to make the tests or of the response of the eye by the visual method. This presents no difficulty, provided measurements for sample and standard are made with the same colorimeter. However, unless only rough approximation is adequate, the color values presented in Table I must be redetermined on the colorimeter at hand if it is desired to compute a given color measurement in terms of the various phenols.

From Table I it is seen that a wide variation exists in relative color intensities produced by the various phenolic compounds; this variation appears to be mainly influenced by the location of the substituted groups on the benzene ring. Of the monohydroxyphenols, the most intense colors are given by those phenols with alkyl groups in the ortho position with respect to the hydroxyl group; the least color is given by the most highly substituted compounds. Between these extremes no simple generalizations are valid. With concentrated phenol mixtures such as cresylic acids obtained from petroleum, the relative color intensity appears to decrease with an increase in average boiling point. With respect to pure phenols, the color intensity is not related linearly to the molecular weight. Of the thirty-six phenolic compounds or mixtures tested, only *p*-hydroxybenzoic acid failed to give a significant color by the proposed method. The cause of this anomaly is unknown at present.

Variation of Color Intensity with Concentration of Phenols. Calibration curves for use with photoelectric colorimeters were prepared for several pure phenols and phenol mixtures by treating various known weights of these compounds according to the proposed procedure. In Figure 1 are presented calibration curves for phenol, *p*-cresol, 2,6-dimethylphenol, 2,4,6-trimethylphenol, and petroleum cresylic acids (boiling range 220–225° C.) obtained with the Spekker absorptiometer using the No. 7 filter (violet) supplied with apparatus. In Figure 2 are presented the calibration curves for phenol, *o*-cresol, *m*-cresol, *p*-cresol, 3,5-dimethylphenol, 2,4-dimethyl-6-*tert*-butyl phenol, *p*-*ter*-butyl catechol, and petroleum cresylic acids (boiling range 220–225° C.), obtained with the Klett-Summerson photoelectric colorimeter using the No. 42 filter (violet) supplied with the apparatus.

With less than 1 mg. of phenol in the final solution substantially straight-line curves were obtained in most cases, indicating that Beer's law applies in this determination. This is particularly true of the calibration curves obtained for the Klett-Summerson colorimeter, which utilizes a light filter transmitting only a narrow band of light in the violet region (approximately 420 mμ). The Spekker light filter transmits some light in regions other than violet.

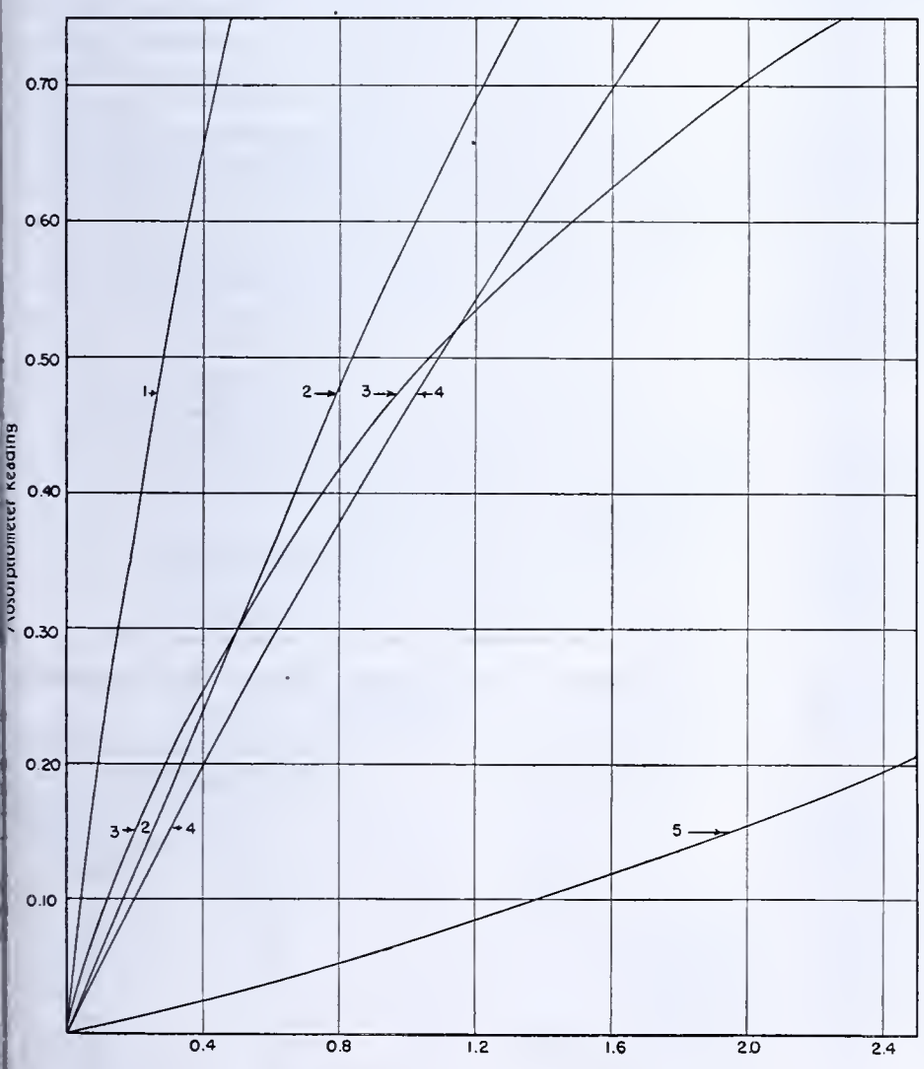

Figure 1. Typical Calibration Curves for Pure Phenols and Phenolic Mixtures

Spekker photoelectric absorptiometer, No. 7 (violet) filter

1. 2,6-Dimethylphenol
2. *p*-Cresol
3. Phenol
4. Petroleum cresylic acid (b.r. 220–225° C.)
5. 2,4,6-Trimethylphenol

Spectral Characteristics of Color Comparison Solutions. When treated by the proposed procedure, various monohydroxyphenols produce color comparison solutions which vary from greenish yellow for phenol and meta-substituted phenols to orange-yellow for other phenols. Dihydroxyphenols and naphthols generally produce brownish-yellow solutions. The various color shades are sufficiently distinct so that an experienced chemist can qualitatively divide them into three groups by visual examination. Figures 3, 4, and 5 give typical spectral transmittance curves for the color comparison solutions produced by a variety of pure phenols and cresylic acid fractions. In most cases the maximum light absorption occurs at wave lengths of 400 to 425 millimicrons. Because of this fact and because the light absorption bands are wide, the color intensity of the comparison solution can be measured by any type of photoelectric colorimeter, provided proper attention is given to the choice of light filter.

Interference by Nonphenolic Compounds. A number of nonphenolic compounds have been tested to determine whether or not they interfere with the phenol determination by exhibiting colors after treatment by the proposed procedure. The data presented in Table II were obtained with exactly 1-mg. samples of various nonphenolic compounds, both with and without blending with 1 mg. of petroleum cresylic acid (boiling range 220° to 225° C.). The values given for relative color intensities are

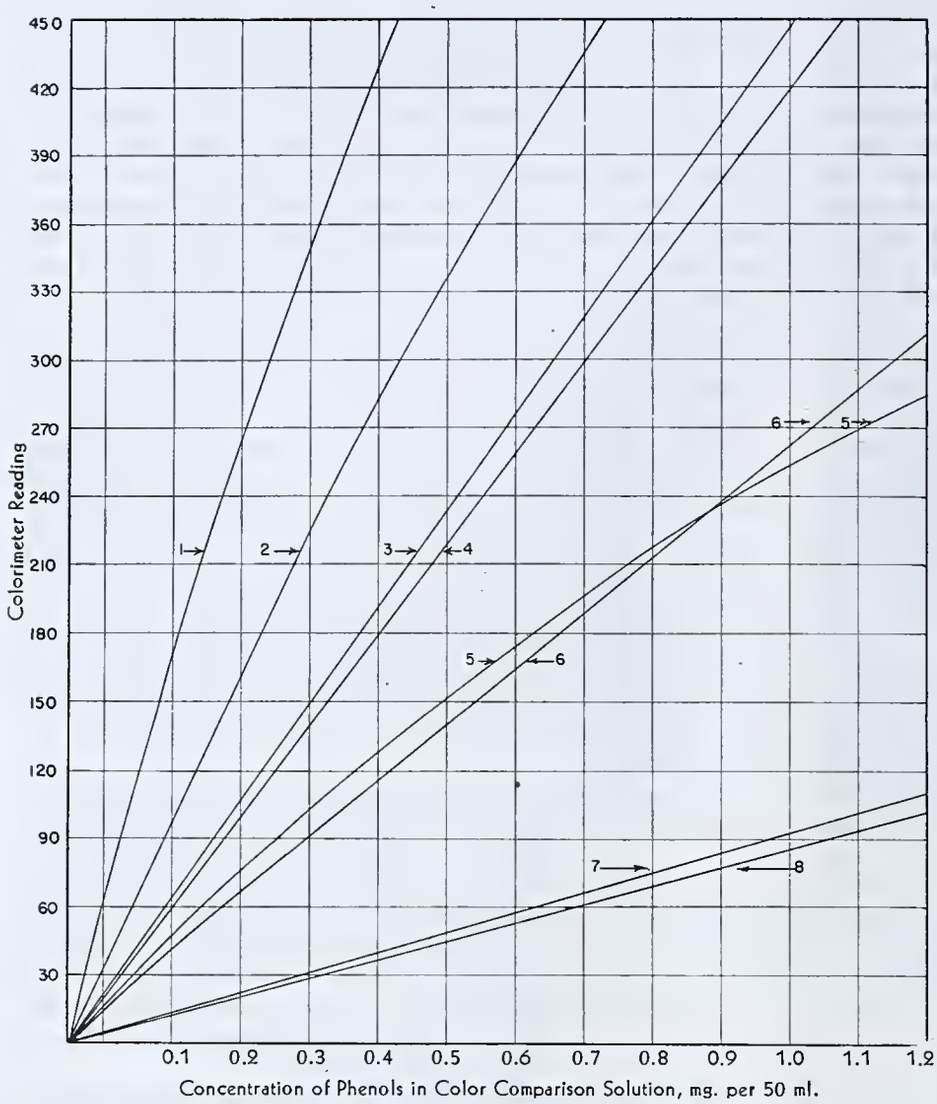

Figure 2. Typical Calibration Curves for Pure Phenols and Cresylic Acid Fractions

Klett-Summerson photoelectric colorimeter, No. 42 (violet) filter

1. *o*-Cresol
2. *m*-Cresol
3. Petroleum cresylic acid (b.r. 220–225° C.)
4. *p*-Cresol
5. 3,5-Dimethylphenol
6. Phenol
7. 2,4-Dimethyl-6-*tert*-butylphenol
8. *p*-*tert*-Butylcatechol

based on the value of unity for the intensity of color produced by exactly 1 mg. of petroleum cresylic acid (boiling range 220° to 225° C.). From these and numerous other tests it was found that with the exception of aniline and xylidine none of the common aliphatic or aromatic nonphenolic compounds give color comparison solutions of the type given by phenols. Therefore it can be assumed that the proposed method is with very few exceptions specific for phenolic materials.

Aniline and xylidine were found to interfere to some extent with the phenol determination; when treated by the proposed direct procedure, they reacted giving color comparison solutions having shade and intensity similar to those obtained with most phenols. However, experiments have shown that the proposed extraction procedure gives satisfactory results in the presence of these aromatic amines, provided the concentration of the phenol is at least twenty times greater than the aniline or xylidine concentration. For samples containing a higher relative concentration of aniline or xylidine, the usual caustic extraction does not generally result in an extract substantially free of the amines. In such cases the following modified procedure gives satisfactory results.

Weigh a sample containing approximately 15 mg. of phenol into a 125-ml. Squibb-type separatory funnel containing 100 ml. of phenol-free technical octane. Add 10 ml. of 5% sulfuric acid and shake the mixture vigorously for 5 minutes. Allow the phases to separate completely and withdraw the lower layer. Add 10 ml. of water to the separatory funnel, shake the mixture for 1 minute, and allow the two phases to separate. Withdraw the lower phase and add it to the lower phase from the first extraction. Repeat the acid and water extractions. Transfer back to the separatory funnel any hydrocarbon phase liberated from the combined acid and water extracts. Analyze the residual extracted hydrocarbon in the separatory funnel by the proposed extraction procedure.

Analyses of samples of some hydrocarbon mixtures, especially cracked gasolines to which known quantities of alkyl phenols had been added, frequently gave low results by the extraction procedure. Subsequent tests showed that the apparent phenol content of such samples tended to decrease with time. Analyses of these samples showed an appreciable concentration of peroxides; the peroxide content was found to increase with time. Apparently the added phenols were oxidized by compounds of the peroxide type resulting from air oxidation of the unsaturated hydrocarbons present. This behavior emphasizes the necessity of analyzing such materials immediately in order to prevent possible changes in phenol content on standing.

Extraction Procedure. The determination by this method of small amounts of phenols present in a hydrocarbon material requires concentration of the phenols by extraction from the hydrocarbon with a minimum quantity of an aqueous, basic solution. Potassium hydroxide solutions were found to be preferable to sodium hydroxide solutions for this purpose because of the generally higher solubility of the potassium phenolates as compared with the sodium phenolates. Using the experimentally chosen extraction procedure contained in this method (which consists of successive extractions with 10 ml. of 10% potassium hydroxide solution, 5 ml. of 10% potassium hydroxide solution, and 5 ml. of water), it was found by analysis of the extracts from technical octane blends that approximately 94% of the added phenol was recovered in the first extract, 6% in the second, and less than 1% in the last. In these tests the sample consisted of 100 ml. of technical octane containing 46 mg. of petroleum cresylic acids (boiling range 220° to 225° C.).

An alternative extraction procedure was tried in which the combined caustic extracts were acidified and the "sprung"

Table II. Relative Color Intensities Produced by Nonphenolic Compounds

Material	Intensity of Color Produced Relative to Color from 1 Mg. of Petroleum Cresylic Acid (b.r. 220–225° C.) 1 mg. of material	1 mg. of material + 1 mg. of petroleum cresylic acid (b.r. 220–225° C.)
Toluene	0.00	1.00
Benzaldehyde	0.00	1.00
Benzyl alcohol	0.00	1.00
Benzoic acid	0.00	1.00
Salicylic acid	0.02	1.00
Phenetole	0.08	1.00
Thiophenol	0.00	1.00
Mixed alkyl thiophenols	0.04	1.00
Mixed naphthenic acids	0.00	1.00
Aniline	0.05	1.08
Pyridine	0.00	1.00
Quinoline	0.00	1.00
Mixed nitrogen bases from petroleum	0.00	1.00
Mixed nonbasic nitrogen compounds from petroleum	0.00	1.00
Methyl ethyl ketone	0.00	1.00

Table III. Influence of Water Content of Reaction Medium on Intensity of Color Produced by Various Phenols

Phenol Present (1 Mg.)	Relative Color Intensity Using 5 Ml. of Acetic Acid Solution of Indicated Water Content					
	0[a]	5% volume	10% volume	20% volume	40% volume	60% volume
Phenol	1.00	1.05	1.00	1.00	0.30	0.20
p-Cresol	1.00	0.77	0.71	0.71	0.71	0.69
o-Cresol	1.00	1.11	1.18	1.29	0.95	0.69
m-Cresol	1.00	1.11	1.14	1.11	0.77	0.44
p-Ethylphenol	1.00	0.80	0.77	0.74	0.74	0.71
m-Ethylphenol	1.00	1.11	1.14	1.14	0.95	0.80
2,4-Dimethylphenol	1.00	1.43	1.54	1.67	1.67	1.67
2,5-Dimethylphenol	1.00	1.33	1.54	1.54	1.11	0.77
2,6-Dimethylphenol	1.00	2.50	3.60	4.40	4.40	4.00
3,4-Dimethylphenol	1.00	1.05	1.08	1.05	0.83	0.77
3,5-Dimethylphenol	1.00	1.15	1.20	1.07	0.86	0.64
2,3,5-Trimethylphenol	1.00	1.20	1.20	1.11	0.97	0.88
Petroleum cresylic acids						
Boiling range 200–220° C.	1.00	1.25	1.33	1.54	1.54	1.43
220–225° C.	1.00	1.33	1.33	1.33	1.43	1.43
220–240° C.	1.00	1.14	1.25	1.33	1.33	1.25
235–290° C.	1.00	1.15	1.25	1.30	1.20	1.15
200–235° C.	1.00	1.18	1.38	1.54	1.54	..

[a] Used as standard of comparison for each phenol only; not intercomparable.

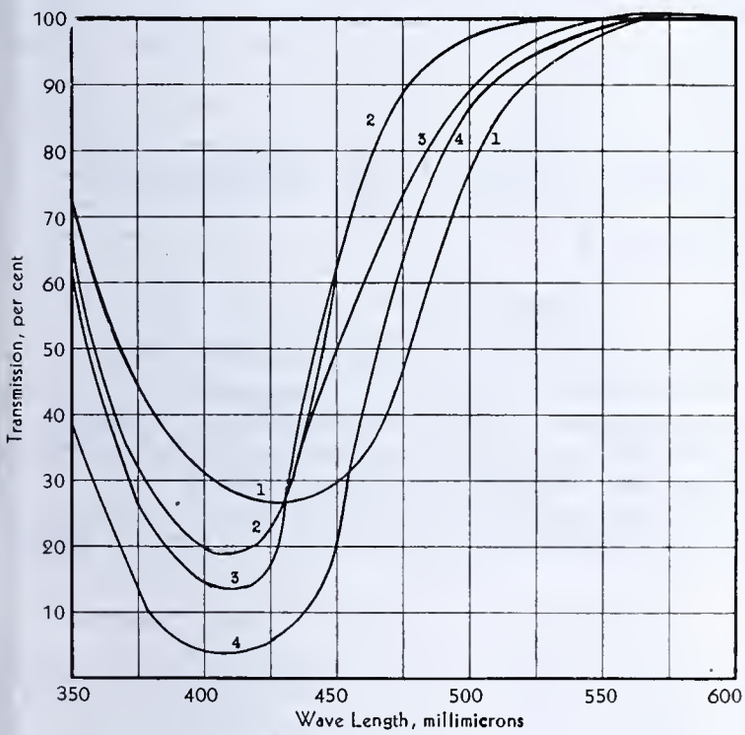

Figure 3. Spectral Characteristics of Color Comparison Solutions for Phenols

Instrument, Coleman spectrophotometer, Model 10S
Cell depth, 13 mm. (square)

1. 0.5 mg. of *p*-cresol
2. 0.5 mg. of *m*-cresol
3. 0.6 mg. of phenol
4. 0.5 mg. of *o*-cresol

phenols extracted with diethyl ether, the ether extracts concentrated to a small volume by evaporation, the phenolic residue dissolved in acetic acid, and the color development carried out by the direct procedure. However, this procedure gave results which were generally about 3% low, indicating incomplete recovery of the phenols.

A variety of caustic solutions were tried as possible extractants for the phenols. None showed any definite advantage over the 10% potassium hydroxide solution initially decided upon. Generally, very concentrated solutions interfered with the color development by precipitating a large amount of salts when the alcohol was added. Modification of the recommended extraction procedure may be required in instances where the samples contain highly substituted alkyl phenols which are not easily extractable by 10% potassium hydroxide solution.

The application of the direct procedure to hydrocarbon samples was found to give low, erratic results even when the sample contained relatively high phenol concentrations. Evidently the acetic acid–potassium acetate solution failed to extract the phenols quantitatively or the color complex was soluble in the insoluble hydrocarbon layer which had to be removed by filtration.

Color Development Procedure. The most critical variable factors involved in the color development procedure were studied to determine their possible effect on the reproducibility of the method and to discover their optimum values. The composition of the reaction medium is an important factor. Variations in the concentration of either water or inorganic salts may have appreciable effects upon the intensity of the color of the final solution. The magnitude of these effects is indicated by the data of Table III. With most phenols variations in water content produced the least effect when 10 to 40% water was initially present; consequently, 20% water seemed most desirable. For the sake of uniformity and convenience the composition of the reaction medium was standardized at the composition corresponding to that obtained in the extraction procedure by diluting the accumulated caustic and water extracts to 100 ml. with glacial acetic acid. Under the conditions of the test the presence of the potassium acetate gave no undesirable effects and did not materially change the color intensity.

For the standard reaction mixture volume of 5 ml. it was found that the quantity of concentrated sulfuric acid could be varied between 3 and 12 drops without noticeable effect; similarly, the volume of saturated sodium nitrite solution could be varied between 1 and 4 drops without changing the result. The quantity of ammonia finally added to the reaction mixture was without effect on the color intensity, provided this amount was at least 175% of that required for neutralization of the acids present. However, different color intensities were produced if care was not taken to keep the mixture cool while neutralizing with ammonia.

The time of standing of the reaction mixture (after addition of the sulfuric acid and sodium nitrite) before neutralization with

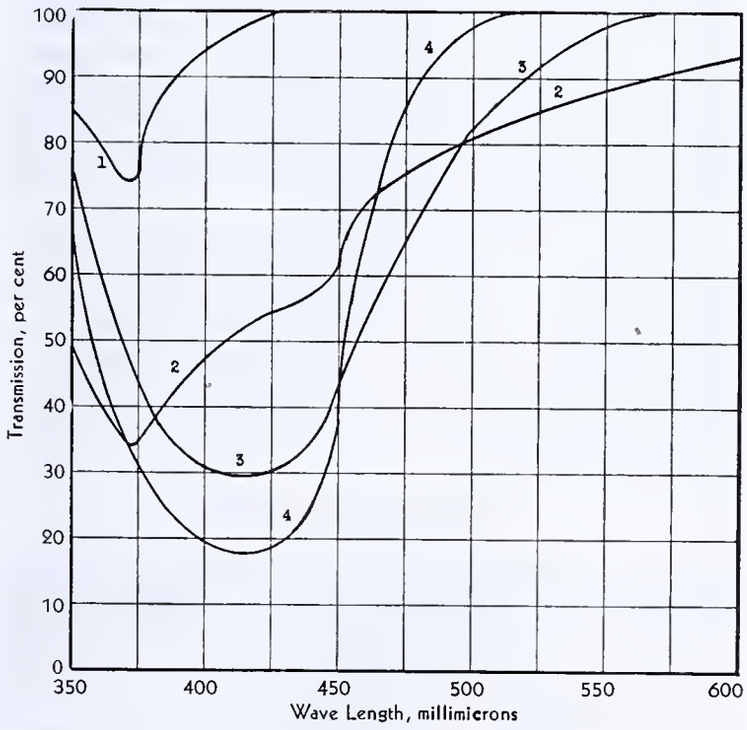

Figure 4. Spectral Characteristics of Color Comparison Solutions for Phenols and Phenolic Mixtures

Instrument, Coleman spectrophotometer, Model 10S
Cell depth, 13 mm. (square)

1. Reagent blank
2. 0.5 mg. of hydroquinone
3. 0.62 mg. of petroleum cresylic acid (b.r. 220–225° C.)
4. 0.5 mg. of resorcinol

Table IV. Influence of Time of Standing before Neutralization on Intensity of Color Produced by Phenols

Phenol present	Weight, Mg.	Net Colorimeter Reading for Indicated Time of Standing before Neutralization[a] 5 min.	10 min.	20 min.	30 min.	40 min.
Phenol	0.20	78.2	114.0	176.5	190.0	196.0
Phenol	0.80	313	495	560	564	580
o-Cresol	0.20	211.0	211.0	213.0	213.0	213.0
p-Cresol	0.20	90.0	92.0	92.0	92.0	92.0
p-Cresol	1.20	540	540	553	553	553
m-Cresol	0.20	162.0	156.0	142.0	142.0	142.0
Petroleum cresylic acid						
Boiling range 200–220° C.	0.20	147.0	148.0	137.5	137.0	132.0
200–225° C.	0.20	136.0	136.0	128.0	128.0	121.0
220–225° C.	0.20	98.8	97.5	93.4	88.2	82.5
220–225° C.	1.20	582	582	535	529	506
220–240° C.	0.20	79.0	79.0	80.0	73.0	73.0
235–290° C.	0.20	54.0	54.5	54.0	50.0	48.0

[a] All solutions allowed to stand 23 to 24 hours after neutralization before measuring color intensity. All measurements with Klett-Summerson photoelectric colorimeter.

Table V. Influence of Time of Standing after Neutralization on Intensity of Color Produced by Phenol Mixtures

Phenol Present	Weight, Mg.	Net Colorimeter Reading for Indicated Time of Standing after Neutralization[a] 0 hour	0.25 hour	1 hour	4 hour	24 hour
Petroleum cresylic acids						
Boiling range 220–225° C.	1.06	0.448	0.460	0.471	0.482	0.484
200–220° C.	1.03	0.602	0.613	0.623	0.629	0.633
200–225° C.	0.97	0.539	0.544	0.559	0.560	0.570
200–235° C.	1.16	0.549	0.558	0.565	0.576	...
220–240° C.	1.03	0.360	0.363	0.371	0.378	0.382
225–245° C.	1.01	0.360	0.363	0.371	0.372	0.383
235–290° C.	1.03	0.260	0.258	0.259	0.259	0.258

[a] All solutions allowed to stand 15 minutes before neutralization. All measurements made by Spekker photoelectric absorptiometer.

ammonia had an important bearing upon the color intensity of the final solution. In Table IV are summarized data showing the observed effects with samples of various phenols when the time of standing before neutralization was varied, other factors being maintained constant. There was no definite trend observed in the time range studied. However, a 15- to 30-minute period of standing before neutralization gave a fairly constant intensity of color for the majority of phenols; with phenol itself a 30- to 45-minute period gave essentially maximum color development. Consequently, these limits were incorporated into the method.

Heating of the reaction mixture was found unnecessary for development of the full color intensity. Tests indicated that the color developed to approximately the same intensity in the same period of time at 40° C. as at 25° C.

The color intensity of the final solution was generally found to increase gradually with time. The data of Table V indicate that a minimum period of standing of 4 hours after neutralization is desirable to avoid minor errors. Experience has indicated that overnight standing is preferable when time permits. Variation in time of standing before or after neutralization did not appear to alter the relative spectral characteristics of the color comparison solutions.

ACCURACY AND PRECISION

The accuracy and precision of the proposed method were determined by analysis (using the extraction procedure) of samples of technical octane to which known amounts of alkyl phenols had been added. These data, presented in Table VI, show that with a satisfactory photoelectric colorimeter results can be obtained by the extraction procedure with a precision of better than ±1% (0.01 mg.). The precision of the direct procedure is generally limited by the repeatability of the photoelectric colorimeter used; with a sensitive colorimeter and selective light filter a precision of ±0.5% (0.005 mg.) is generally obtained.

The systematic error is less than 2% of the actual concentration and is generally a negative error. However, the accuracy of the method is dependent largely upon choosing as a standard a phenol material which closely corresponds to that present in the sample being analyzed.

DISCUSSION

The proposed method has been found applicable to the determination of naphthols, monohydroxyphenols, and dihydroxyphenols in a large variety of substances; very few nonphenolic compounds interfere with the method. Through use of either the direct or extraction procedure satisfactory analyses have been obtained with many different types of samples, including gasoline, kerosene, fuel oil, thinners, hydrocarbon solvents, naphthenic acids, concentrated phenols, alcohols, polymers, chemical preparations, aqueous treating solutions, and other similar materials.

With samples containing a phenol or a mixture of phenols which has been partially identified the proposed method will give results with accuracy and precision comparable to most photoelectric methods. As a method for the analysis of a complex group of phenolic compounds, such as those occurring in petroleum products, the accuracy of the method depends upon the proper choice of standard. In most cases the results need only be relatively accurate, a condition which can be realized by use of an arbitrarily chosen standard such as pure phenol or *o*-cresol. In cases where the identity of the phenol is unknown yet accurate results are needed, it is necessary to isolate a small quantity of the phenol or phenol mixture. With aqueous solutions this can be accomplished by adjusting the solution to contain a slight excess of strong base (pH 12), filtering, adjusting the filtrate to the phenolphthalein end point (pH 8.5), and separating the phenols by centrifuging. With nonaqueous solutions, a quantity of sample is extracted with caustic solution and the phenols are sprung from the extract in the manner described.

Since only a few milligrams of the concentrated phenol mixture are required and quantitative recovery is not essential, isolation

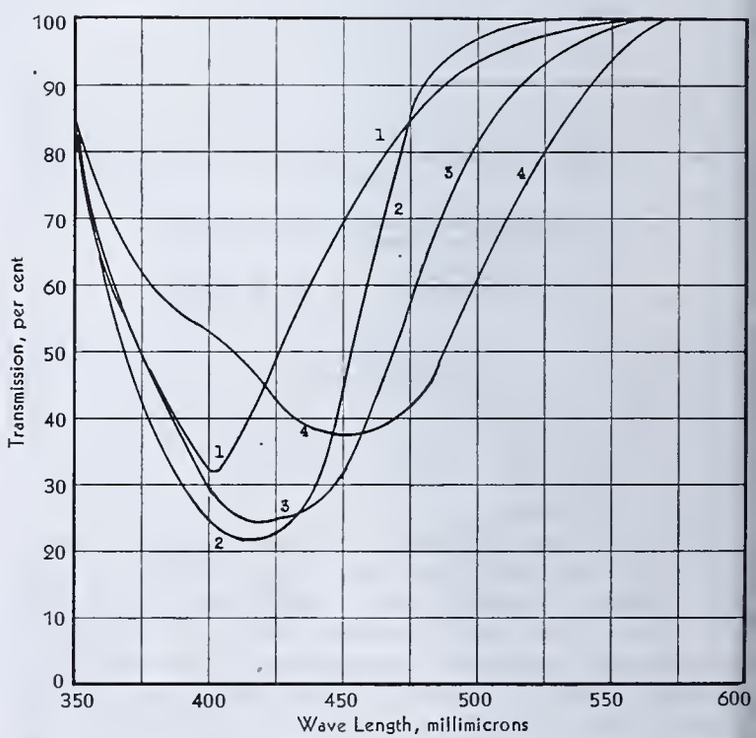

Figure 5. Spectral Characteristics of Color Comparison Solutions for Isomeric Dimethyl Phenols

Instrument, Coleman spectrophotometer, Model 10S
Cell depth, 13 mm. (square)

1. 0.58 mg. of 3,5-dimethylphenol
2. 0.54 mg. of 2,5-dimethylphenol
3. 0.54 mg. of 2,3-dimethylphenol
4. 0.54 mg. of 2,4-dimethylphenol

Table VI. Accuracy and Precision of Proposed Method

Petroleum Cresylic Acids (B.R. 220–225° C.) Added	Cresylic Acids Found[a]	Recovery
Mg./100 ml. technical octane	*Mg.*	%
0.0	0.0	...
10.6	10.5	98.8
10.6	10.4	97.9
10.6	10.3	97.2
10.6	10.3	97.2
10.6	10.5	98.8
10.6	10.5	98.8
	Systematic error	−1.9
	Standard deviation	±0.7
92.2	92.4	100.2
92.2	92.2	100.0
92.2	90.9	98.6
92.2	91.5	99.2
92.2	90.8	98.5
92.2	91.3	99.0
	Systematic error	−0.7
	Standard deviation	±0.6

[a] Spekker photoelectric absorptiometer.

of the phenols presents only a minor problem. In any application it must always be realized that the colorimeter response per milligram of phenol is a specific property of that phenol or phenol mixture only—that is, the amount of color produced by the various phenols varies markedly. Accuracy of the method will depend upon the proper choice of a standard.

The experimental work reported in this paper demonstrates the wide scope and versatility of the method. It has found extensive use in research, control, and specification analytical work involving various types of materials and phenolic compounds.

ACKNOWLEDGMENT

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Analysis of Ternary Mixtures of Methylcyclohexane-Toluene-Aniline

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A method of analyzing the ternary mixture methylcyclohexane-toluene-aniline has been developed. The refractive index of the ternary mixture and of the residual binary hydrocarbon after removing the aniline by acid extraction completely defines the ternary system. With the aid of an experimentally established curve, the ternary analysis can be found.

THE analysis of a binary liquid mixture can often be made by measuring one physical property such as refractive index or density, when a second variable such as temperature is fixed. The introduction of a third component complicates the problem and necessitates fixing another variable to permit analysis by the measurement of one physical property. Unfortunately, fixing another variable is not easy, since both temperature and pressure (1 atmosphere) have already been fixed for the two-component system.

Varteressian and Fenske (*2*) analyzed mixtures of *n*-heptane–methylcyclohexane-aniline by means of refractive index measurements for the special case of liquid-liquid extraction where the solution was saturated and in equilibrium with a second immiscible solution at a given temperature and pressure, and also applied this method to the system benzene–ethyl alcohol–water (*1*) under the same conditions. The method of analysis described in this paper is concerned with the case where the solution is not saturated under specified conditions, but exists as a completely miscible solution.

PROPERTIES OF MATERIALS USED

The methylcyclohexane was the best commercial grade furnished by the Rohm and Haas Company of Philadelphia. It was extracted with concentrated sulfuric acid, washed, dried, and then fractionated in a laboratory column of approximately 35 theoretical plates. The best cuts from the fractionation were combined and used in this work. The purified material had the following properties:

50% boiling point at 760 mm., ° C.	100.90
0 to 50% boiling point spread, ° C.	0.0
Density, d_4^{20}	0.7695
Refractive index, n_D^{20}	1.42310

The toluene was also purified before use. The best commercial grade of nitration toluene furnished by the Barrett Company, Frankford, Pa., was fractionated in a large column of approximately 75 theoretical plates. The best cuts from the fractionation were sulfonated with concentrated sulfuric acid and separated from impurities by steam-distillation, after which the toluene was regenerated by hydrolysis. The regenerated toluene was then first fractionated to remove any by-product of hydrolysis. The purified material had the following properties:

50% boiling point at 760 mm., ° C.	110.65
0 to 50% boiling point spread, ° C.	0.0
Freezing point, ° C.	−95.2
Density, d_4^{20}	0.8672
Refractive index, n_D^{20}	1.49685

The aniline was the water-white commercial product of the Dow Chemical Company. Before use, it was dried over Baker's sodium hydroxide pellets and then subjected to three successive simple distillations, discarding a generous portion of the fore-

Table I. Analysis of Mixtures of Methylcyclohexane-Toluene-Aniline

(Using refractive index of binary hydrocarbon mixture and of ternary mixture)

	Composition of Test Mixtures, Weight Per Cent								
	Gravimetric Composition			By Analysis			Per Cent Error		
	M[a]	T	A	M	T	A	M	T	A
1	8.24	36.80	54.95	8.3	37.1	54.6	+0.73	+0.81	−0.64
2	20.90	23.52	55.60	20.8	23.4	55.8	−0.48	−0.51	+0.36
3	33.80	9.60	56.70	33.4	9.3	57.3	−1.18	−3.13	+1.06
4	13.20	14.85	71.90	13.1	14.6	72.3	−0.76	−1.68	+0.56
5	36.70	40.70	22.60	36.8	40.4	22.8	+0.27	−0.74	+0.88

[a] M = methylcyclohexane, T = toluene, A = aniline.

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Table II. Composition of Hydrocarbon Portion of Ternary Mixtures, Methylcyclohexane-Toluene-Aniline

(Expressed as mole per cent methylcyclohexane on a binary hydrocarbon basis)

Gravimetric Composition	Composition by Refractive Index, Analysis after Removal of Aniline by Dilute HCl[a]	Per Cent Error
17.4	17.5	0.57
45.5	45.4	−0.22
76.75	77.1	0.46
45.2	45.5	0.66
45.9	46.05	0.33

[a] One volume of concentrated hydrochloric acid diluted to 5 volumes with distilled water.

runnings and the residue. The purified aniline had the following properties:

Density, d_4^{20}	1.0219
Refractive index, n_D^{20}	1.5863

Since the system being analyzed consists of one phase and three components, the phase rule, $P + V = C + 2$, indicates that there are four variables. Two variables, temperature and pressure, can be easily fixed. When a third variable is fixed, measurement of a fourth variable will define the system. The third variable fixed is the ratio of methylcyclohexane to toluene or the composition of the hydrocarbon portion of the mixture on a solvent-free basis.

Table III. Refractive Index *vs.* Composition of Methylcyclohexane-Toluene-Aniline Mixtures with Mole Per Cent Methylcyclohexane in Toluene as Parameter[a]

Wt. % Aniline	n_D^{20} Ternary Mixture	Wt. % Aniline	n_D^{20} Ternary Mixture
0.0 Mole %[a]		9.9 Mole %	
0.00	1.4969	0.00	1.4874
10.40	1.5048	9.96	1.4957
20.80	1.5132	20.20	1.5048
31.35	1.5226	29.70	1.5136
40.60	1.5306	40.80	1.5241
50.40	1.5394	50.35	1.5334
60.30	1.5486	60.70	1.5439
70.70	1.5581	69.90	1.5535
80.20	1.5669	80.15	1.5664
20.2 Mole %		30.3 Mole %	
0.00	1.4784	0.00	1.4700
10.20	1.4880	11.46	1.4809
20.70	1.4981	20.25	1.4897
30.30	1.5076	30.20	1.5002
40.40	1.5181	40.40	1.5115
50.85	1.5291	51.20	1.5235
60.55	1.5398	60.10	1.5343
70.40	1.5512	70.50	1.5470
77.30	1.5582	80.20	1.5591
40.0 Mole %		49.05 Mole %	
0.00	1.4621	0.00	1.4552
10.23	1.4730	10.50	1.4660
19.98	1.4831	20.15	1.4769
29.95	1.4944	29.90	1.4888
40.50	1.5070	40.45	1.5020
50.60	1.5188	50.65	1.5140
59.80	1.5300	61.50	1.5289
70.00	1.5440	70.70	1.5418
80.30	1.5586	79.80	1.5550
59.4 Mole %		70.1 Mole %	
0.00	1.4479	0.00	1.4408
9.90	1.4583	10.20	1.4520
19.70	1.4698	29.80	1.4762
30.25	1.4824	26.45	1.4722
40.40	1.4958	40.70	1.4905
50.10	1.5090	50.60	1.5046
60.20	1.5232	60.40	1.5191
70.20	1.5380	69.20	1.5335
80.60	1.5545	80.20	1.5515
78.4 Mole %		90.0 Mole %	
0.00	1.4355	0.00	1.4285
10.90	1.4472	10.50	1.4400
19.50	1.4580	20.40	1.4505
30.30	1.4720		
40.50	1.4865	Phase separation occurred here	
49.80	1.5000		
69.90	1.5325		
79.90	1.5494		

[a] Mole % methylcyclohexane in toluene on binary basis.

ANALYTICAL PROCEDURE

The refractive index, n_D^{20}, of the ternary mixture is read. The aniline is then removed from the mixture by extraction with dilute hydrochloric acid (1 volume of concentrated acid diluted to 5 volumes with distilled water). The refractive index, n_D^{20}, of the hydrocarbon after this extraction is also read. From the latter datum, the composition of the hydrocarbon mixture can be obtained by means of a refractive index–composition chart. This composition and the refractive index of the ternary mixture are used in conjunction with the curves in Figure 1 to obtain the weight per cent of aniline in the ternary mixture. From this value and the composition of the hydrocarbon portion of the mixture, the over-all composition is calculated.

To check the accuracy of this method of analysis, five mixtures of methylcyclohexane-toluene-aniline were made up gravimetrically and analyzed. The gravimetric analyses and the experimental analyses of the mixtures are listed in Table I. The percentage error is based upon the gravimetric analysis as correct. Examination of the analyses shows good agreement in most cases, while no value is greatly in error.

Two sources of error exist in these analyses: the accuracy with which the refractive index of the ternary mixture can be read, and the accuracy of the hydrocarbon analysis after the aniline has been removed by dilute hydrochloric acid extraction. A study of the data in Table II will show that in almost every case the accuracy of the hydrocarbon analysis is within the accuracy of the refractive index measurements. Here again the percentage error is based upon the gravimetric composition.

The technique employed in obtaining the curves in Figure 1 was as follows:

A binary hydrocarbon mixture of methylcyclohexane-toluene of known composition was weighed out and combined with weighed amounts of aniline. The refractive indexes of these mixtures were read and a curve of refractive index versus weight per cent aniline was plotted with mole per cent methylcyclohexane in the binary hydrocarbon mixture as parameter. Ten points were obtained to establish each curve, and nine such curves were deter-

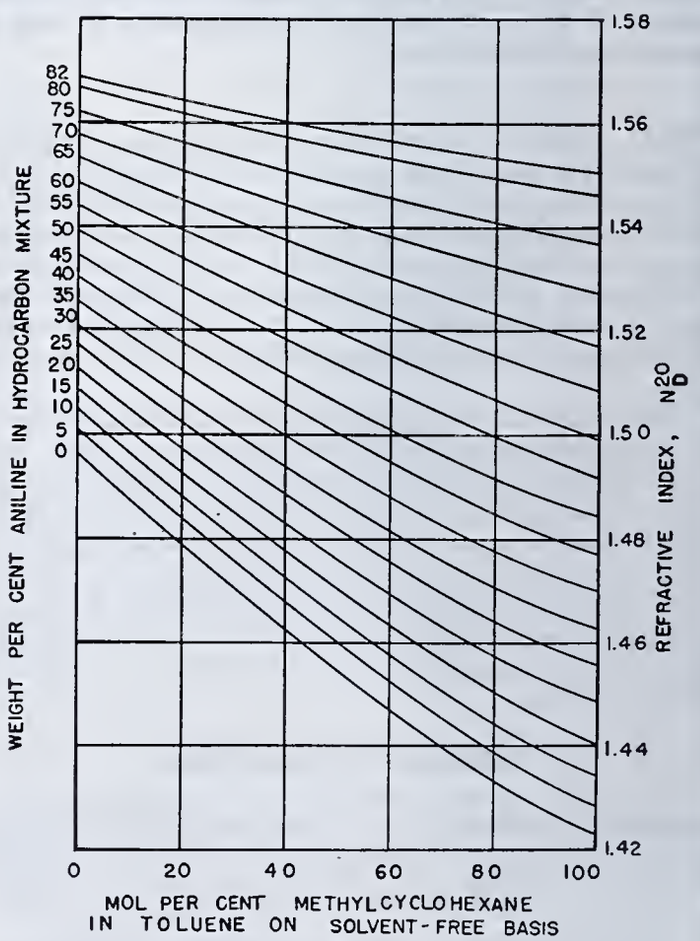

Figure 1. Analysis of Ternary Mixtures

Table IV. Refractive Index, Mole Per Cent Hydrocarbon Composition Data for Figure 1 for System Methylcyclohexane-Toluene-Aniline

Mole % Methylcyclohexane[a]	0	9 9	20.2	30.3	40.0	49.05	59.4	70.1	78.4	90.0
Wt. % aniline in ternary	Refractive Index of Ternary Mixture									
0	1.4969	1.4874	1.4784	1.4700	1.4621	1.4552	1.4479	1.4408	1.4355	1.4285
5	1.5006	1.4914	1.4831	1.4747	1.4671	1.4601	1.4530	1.4461	1.4406	1.4337
10	1.5045	1.4957	1.4878	1.4795	1.4723	1.4653	1.4585	1.4517	1.4462	1.4394
15	1.5085	1.5000	1.4926	1.4845	1.4777	1.4709	1.4642	1.4577	1.4522	1.4455
20	1.5125	1.5046	1.4974	1.4896	1.4831	1.4767	1.4701	1.4639	1.4585	1.4522
25	1.5168	1.5091	1.5024	1.4947	1.4887	1.4827	1.4761	1.4700	1.4650	....
30	1.5211	1.5138	1.5073	1.4999	1.4944	1.4889	1.4823	1.4765	1.4717	....
35	1.5255	1.5185	1.5124	1.5053	1.5000	1.4950	1.4887	1.4829	1.4786	....
40	1.5300	1.5233	1.5175	1.5108	1.5059	1.5013	1.4953	1.4895	1.4857	....
45	1.5345	1.5281	1.5227	1.5164	1.5119	1.5076	1.5020	1.4964	1.4929	....
50	1.5391	1.5330	1.5281	1.5221	1.5181	1.5140	1.5088	1.5037	1.5004	....
55	1.5437	1.5381	1.5336	1.5280	1.5243	1.5205	1.5158	1.5111	1.5081	....
60	1.5483	1.5432	1.5392	1.5340	1.5308	1.5271	1.5229	1.5188	1.5160	....
65	1.5528	1.5483	1.5449	1.5401	1.5373	1.5339	1.5302	1.5267	1.5242	....
70	1.5574	1.5537	1.5506	1.5463	1.5441	1.5409	1.5377	1.5348	1.5326	....
75	1.5620	1.5589	1.5564	1.5526	1.5509	1.5480	1.5455	1.5429	1.5410	....
80	1.5667	1.5641	1.5622	1.5589	1.5578	1.5553	1.5534	1.5512	1.5496	....
82	1.5686	1.5663	1.5645	1.5615	1.5605	1.5583	1.5565	1.5545	1.5531	....

[a] Composition of hydrocarbon mixture on binary basis.

mined. Curves were easily fitted to the experimental points which are given in Table III. Since the weight per cent aniline was the desired parameter in this work, a cross plot was made, taking points from the curves at each 5 weight per cent aniline. These data, given in Table IV, provided nine points to establish each curve in Figure 1.

The curves in Figure 1 are not straight lines as is sometimes assumed. Charts similar to Figure 1 can be constructed completely on a mole basis or on a weight basis by converting the data in Table IV to the desired values.

ACKNOWLEDGMENT

Acknowledgment is due D. Quiggle, S. Lawroski, and members of the staff of the Petroleum Refining Laboratory for suggestions and help in the purification of the materials used.

CONCLUSIONS

Ternary mixtures of methylcyclohexane-toluene-aniline can be analyzed rapidly and with good accuracy by reading the refractive index of the ternary mixture, followed by reading the refractive index of the hydrocarbon portion of the mixture after the aniline has been removed.

The same procedure may be useful for analyzing other types of hydrocarbon-solvent systems.

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Rapid Photometric Determination of Iron in Aluminum Alloys

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A method for the photometric determination of iron in aluminum alloys is based on the reaction of ferrous iron and 1,10-phenanthroline. The information given in this report is concerned with the formation, stability, and reproducibility of the reaction of ferrous iron and 1,10-phenanthroline. The method is rapid and the accuracy obtained is ±0.05% of the amount present.

A NUMBER of papers (*1*, *3*, *5*, *7*) have described the use of 1,10-phenanthroline in the photometric determination of iron in foods, iron ores, and biological materials.

Smith and Richter summarized work done with 1,10-phenanthroline up to 1944 (*6*).

Blau gave a description of the properties of 1,10-phenanthroline along with the method of preparation (*1*). Walden, Hammett, and Chapman showed that the complex ion formed with ferrous iron and 1,10-phenanthroline has a high oxidation potential and may be used as an indicator in certain oxidimetric procedures (*7*). Saywell and Cunningham developed a photometric method for determining small amounts of iron in fruit juices (*5*). In the field of metallurgical analysis, Mehlig and Hulett described a method for the photometric determination of iron in iron ores (*3*).

The purpose of the work described in this paper was the development of a rapid and accurate method for the determination of iron in aluminum alloys based upon the formation of ferrous phenanthroline complex.

EXPERIMENTAL WORK

The reaction of 1,10-phenanthroline and ferrous iron is the basis for the work described in this paper. At the time of the investigation, there were no known published papers describing the use of 1,10-phenanthroline in the determination of iron in aluminum alloys.

The reaction of 1,10-phenanthroline and ferrous iron produced an orange colored solution, the color intensity varying with the iron content.

Methods for dissolving aluminum were investigated. Hydrochloric acid (1 to 1) was found to be the best solvent for this purpose; it also aided in the removal of interfering ions, which are insoluble in hydrochloric acid. If sodium hydroxide were used, hydroxylamine hydrochloride when added would act as an oxidizer of iron instead of a reductant (*4*).

The following were the available reductants: 10% aqueous solution of hydroxylamine hydrochloride, 0.25 molar solution of stannous chloride, 10% solution of sodium sulfite, formaldehyde, and hydroquinone. The 10% solution of hydroxylamine hydrochloride proved to be the best, as appreciable errors showed up when the other reductants were used. This was due to the complexes formed with iron which kept the 1,10-phenanthroline from reacting completely with it.

All spectrophotometric measurements were made with the Coleman Universal spectrophotometer Model 11; the cell thickness for this instrument is 13 mm. For routine work the Fisher AC Model electrophotometer may be used with a filter of 490 mμ; the cell thickness is 23 mm.

To determine the wave length which would produce the maximum absorption, a representative concentration of the solution was examined at a series of wave lengths. The peak of absorption when using the Coleman spectrophotometer is at 490 mμ. The representative sample used contained 0.50% iron.

A series of photometric measurements showed that 15 minutes after the 1,10-phenanthroline was added the color became stable and remained so for at least 48 hours. The tests were discontinued at the end of this period.

Very few ions interfere with the determination of iron in aluminum alloys. Fortune and Mellon made an exhaustive general study of ions which interfere with the reaction of ferrous iron and 1,10-phenanthroline. The writer found that silicon, copper, and bismuth interfere with the determination of iron in aluminum alloys (*2*), but these elements are insoluble in hydrochloric acid and, therefore, can be removed by filtration. Zinc forms a precipitate with 1,10-phenanthroline. With small amounts of zinc, it was possible to prevent appreciable interference by adding a slight excess of 1,10-phenanthroline. Unless the concentration of zinc was greater than 10 parts per million it caused no appreciable error. The absence of interfering ions makes the method ideal for rapid, routine work.

REAGENTS REQUIRED

Hydrochloric Acid (1 to 1), 1000 ml. of distilled water and 1000 ml. of c.p. hydrochloric acid (specific gravity 1.19).

Hydroxylamine Hydrochloride (10%). Dissolve 10 grams of c.p. hydroxylamine hydrochloride crystals in 100 ml. of distilled water. (Store in refrigerator. Do not use if the solution has a brown color.)

1,10-Phenanthroline (0.25%). Dissolve 0.500 gram of c.p. 1,10-phenanthroline monohydrate crystals in 150 ml. of boiling distilled water. Cool, transfer to a 200-ml. volumetric flask, and dilute with distilled water. (Store in refrigerator. Do not use if the solution has a brown color, as this indicates decomposition.)

Standard Iron Solution. Dissolve 1.000 gram of pure iron wire in 50 ml. of concentrated hydrochloric acid. Transfer to a 1000-ml. volumetric flask. Dilute to the mark with distilled water (1 ml. = 0.1% iron or 0.001 gram of iron).

Table I. Comparison of 1,10-Phenanthroline Photometric Method and Other Methods

Sample No.	Other Methods[a] %	1,10-Phenanthroline Photometric Method %
114B	0.66	0.66
85	0.38	0.39
86B	1.52	1.53
4064	0.33	0.33
3044	0.68	0.67
2624	0.80	0.80
9193	0.32	0.33
N1218	0.98	0.98
N1223	1.02	1.00

Each value is an average of 12 determinations.

[a] Permanganate and dichromate methods.

METHOD

Dissolve a 0.500-gram sample of aluminum in 30 ml. of hydrochloric acid (1 to 1), using a 250-ml. beaker and heating if necessary. Filter into a 500-ml. volumetric flask, using Whatman No. 41 filter paper. Wash five times with hot distilled water, cool, and dilute to mark with distilled water. Pipet 10 ml. of solution into a 100-ml. volumetric flask if the sample contains up to 0.50% iron. Pipet 5 ml. of solution into a 100-ml. volumetric flask if the sample contains over 0.50% iron. Add 1 ml. of hyroxylamine hydrochloride (10%) and mix. Add approximately 70 ml. of distilled water and mix. Add 10 ml. of 1,10-phenanthroline (0.25%) and mix. Dilute to the mark with distilled water, shake thoroughly, and let stand for at least 15 minutes. Using the Coleman spectrophotometer, set the wave length dial at 490 and measure the color density of the solution. If a Fisher electrophotometer is used, a 490 mμ filter is required. Use distilled water as reference solution.

A shortage of standard samples made it necessary to develop a method by which a pure iron solution could be used. This was possible because of the absence of interfering elements. A 1.000-gram sample of pure iron wire was dissolved in 50 ml. of concentrated hydrochloric acid, then transferred to a 1000-ml. volumetric flask and diluted to the mark with distilled water (1 ml. = 0.1% iron or 0.001 gram of iron).

All results obtained in this study were calculated on the basis of a pure iron solution standard (Table I). The percentage error was approximately ±0.05% of the amount present.

Beer's law was followed by the color system, as shown by the straight line obtained when the readings of the observed transmittancies at 490 mμ for the solution containing up to 5.00% iron were plotted logarithmically.

DISCUSSION

The formation of the complex when ferrous iron reacts with 1,10-phenanthroline is represented by the radical: $(C_{12}H_8N_2)_3$-Fe^{++}. This is an orange-colored compound, formed when 1 molecule of ferrous iron combines with 3 molecules of 1,10-phenanthroline.

The basic reaction of hydrochloric acid and the sample does not form any ferric iron, but, upon heating, some of the ferrous iron may be converted to the ferric state. The addition of the reductant, hydroxylamine hydrochloride, converts this ferric iron to ferrous. The reverse occurred when the sample of aluminum was dissolved in sodium hydroxide; instead of hydroxylamine hydrochloride acting as a reductant, it became an oxidizer of iron. This method compares favorably with other methods of determining iron in aluminum alloys.

An important advantage over other photometric methods for determination of iron is that the pH need not be regulated closely. However, the ferrous color will not develop at a pH much below 2, and the reduction of iron with hydroxylamine is slow at a pH much above 3. The color formation occurs in the acid solution, eliminating the difficulties usually caused by precipitation of metal hydroxides and hydrated oxides in alkaline solution. Another advantage is freedom from interference of diverse ions.

SUMMARY

The reaction of 1,10-phenanthroline and ferrous iron produces an orange-colored complex. Any ferric iron present is reduced with hydroxylamine hydrochloride.

Very few ions interfere with the color reaction. Silicon, copper, and bismuth are the most common elements present in aluminum alloys which interfere with the formation of the ferrous-phenanthroline complex, but they are eliminated by filtration because they are insoluble in the solvent for aluminum.

Tests prove that the color reaction is stable for at least 48 hours, thus confirming the observations of other workers.

The wave length of 490 mμ produces the maximum absorption of these instruments.

Representative samples, analyzed and plotted logarithmically, prove Beer's law holds for the concentrations employed.

An accuracy of ±0.05% is possible with this method. It is more rapid than the standard volumetric or gravimetric methods for determining iron in aluminum alloys.

ACKNOWLEDGMENT

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Chemical Determination of Vitamin A in Dried Whole Eggs

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A chemical method is described for the determination of vitamin A in dried whole eggs by the use of chromatographic adsorption. After the sample is hydrolyzed with alkali and extracted with ethyl ether, the unsaponifiable fraction is adsorbed on a column of calcium hydroxide and the β-carotene, cryptoxanthol, vitamin A, luteol, and zeaxanthol bands are allowed to separate. The provitamins are eluted and determined separately. Antimony trichloride reagent is then added to the combined vitamin A and provitamin A eluates and the amount of blue color determined in a photoelectric colorimeter. Data presented on nine samples of dried eggs show that the values obtained by the chemical method agree within 10% with those obtained by bioassay.

ATTEMPTS to use published chemical methods (*5*, *7*) for the determination of vitamin A in dried eggs in this laboratory failed to give values in agreement with those obtained by bioassay. Investigation showed that differences resulted from poor extractability of the vitamin and errors caused by the presence of large amounts of carotenols for which arbitrary corrections were not reliable.

When extraction of dried eggs in the Waring Blendor was tried as outlined in the method of Schrenk, Chapin, and Conrad (*7*), a large amount of vitamin A remained in the residue. The residue was hydrolyzed with alkali and extracted with ethyl ether, after which the light absorption of the extract was measured at 326 and 450 millimicrons. The values obtained indicated the presence of substantial amounts of both vitamin A and carotenoids. Values obtained by the Carr-Price reaction further substantiated the presence of vitamin A.

Bioassays of the extracted residue provided further proof that ethyl ether extraction of the whole egg does not remove vitamin A quantitatively. In this experiment about 200 grams of a dried egg sample were extracted with ethyl ether in a large Soxhlet extractor for 24 hours. The residue showed a biological potency equivalent to 14.5 I.U. of vitamin A per gram.

Various solvents were used in attempts to remove the vitamin A quantitatively. A 45-gram sample of dried egg was extracted with about 250 ml. of solvent in a Soxhlet extractor. To test the completeness of extraction, a sample of the residue was hydrolyzed with alkali, the unsaponifiable matter was extracted with ethyl ether, and the amount of vitamin A was determined by the Carr-Price reaction. The results (Table I) showed that ethyl ether was the poorest solvent of those tried for the extraction of vitamin A. However, the solvents which were most effective for the extraction had relatively high boiling points, and prolonged heating resulted in darkening of the extract.

Large amounts of carotenols in the dried eggs were shown to cause substantial errors when attempts were made to determine the vitamin A by means of the Carr-Price colorimetric procedure as modified by Koehn and Sherman (*5*). Klose, Jones, and Fevold (*4*) reported satisfactory agreement of results obtained by this method with those shown by bioassay. When this procedure was used with the total unsaponifiable extract the values were consistently higher than those with the bioassay procedure (Table III, column 3). Attempts to employ an arbitrary factor to correct for carotenoids were unsuccessful.

Reliable results were obtained by chromatographic separation of the unsaponifiable extract obtained after direct alkaline hydrolysis of the whole egg. Gillam and Heilbron (*2*) had shown that the vitamin A of egg yolk could be separated from xanthophylls by adsorption on calcium carbonate and elution with petroleum ether–benzene. However, no attempt at quantitative separation was made.

Preliminary trials in this laboratory revealed that calcium hydroxide was a better adsorbent than calcium carbonate for the chromatographic separation of the carotenoids of eggs. When the unsaponifiable extract was adsorbed from a petroleum ether solution and the column developed with a mixture of 60% benzene and 40% petroleum ether a sharp separation was obtained. The carotenoids were eluted from the column in the following order: β-carotene, cryptoxanthol, vitamin A, luteol, and zeaxanthol. The identity of β-carotene, cryptoxanthol, and luteol was established by comparing light absorption curves (Beckman spectrophotometer, Model DU) with the curves of the pure materials (*8*). The zeaxanthol band contained large amounts of isomerized material as indicated by comparison with a curve of pure zeaxanthol. The amounts of β-carotene, cryptoxanthol, and total carotenoids agreed well with the values obtained by Schrenk *et al.* (*7*) (Table III). A satisfactory light-absorption curve on the vitamin A eluate was not obtained. Contributing to this failure was a contaminant dissolved from the calcium hydroxide and Hyflo Super-Cel which absorbed in the region of 310 to 370 millimicrons. The presence of this material was shown by allowing the benzene–petroleum ether solution to run through fresh adsorbent and determining the light absorption of the percolate. This material gave no color with antimony trichloride but attempts to remove it completely before chromatographing were unsuccessful.

Preliminary attempts to determine vitamin A concentration by direct spectrophotometric measurements on the eluate were discouraging. This was consistent with the observations of Hauge, Zscheile, Carrick, and Bohren (*3*) who found that the curves given by the total carotenoids of fresh eggs as well as dried eggs were very different from that of vitamin A alcohol, both in position of maximum and in shape. Denton, Cabell, Bastron, and Davis (*1*) found they could not determine vitamin A in dried eggs spectrophotometrically because impurities absorbing in the region of the vitamin A maximum did not remain constant during storage.

Adsorption and elution of vitamin A from the calcium hydroxide chromatogram were shown to be satisfactory (Table II). Crystalline vitamin A alcohol and the unsaponifiable fraction from standard U.S.P. reference cod liver oil were used in these tests. In each case the vitamin was adsorbed from petroleum ether solution and eluted with benzene–petroleum ether. The solvent was removed from the eluate, the vitamin A redissolved in chloroform and treated with antimony trichloride reagent,

Table I. Efficiency of Solvents in Extraction of Vitamin A from Dried Whole Egg[a]

Solvent	Extraction Time	Per Cent of Initial Weight of Egg Extracted	Vitamin A Content of Residue
	Hours		*I.U./gram*
Ethyl ether	30	38.0	9.3
	89	41.7	9.7
Ethyl alcohol	42	51.0	2.3
Absolute alcohol	89	51.5	2.3
80% benzene, 20% alcohol	42	50.0	2.0
80% benzene, 20% absolute alcohol	89	51.0	2.3
Carbon tetrachloride	94	43.9	2.0
1,2-dichloroethane	94	46.5	1.5
Trichloroethylene	42	45.5	3.7

[a] Vitamin A content by bioassay, 60 I.U. per gram.

[1] Present address, U. S. Army Medical Nutrition Laboratory, Chicago 9, Ill.

and the light transmission determined with an Evelyn photoelectric colorimeter. Further studies showed that vitamin A could be separated from luteol. This pigment followed vitamin A elosely on the chromatogram.

METHOD

For the hydrolysis, a 5.0-gram sample is weighed into a 125-ml. Erlenmeyer flask, and 20 ml. of absolute methanol and 5 ml. of saturated aqueous potassium hydroxide are added. The contents are stirred with a glass rod until eomplete suspension of the sample is effected. The flask is then heated on a steam bath for 10 minutes or until the dried egg particles are disintegrated. The hydrolyzate is then cooled and transferred with 70 ml. of water to a 500-ml. separatory funnel. The first extraction is made with 35 ml. of peroxide-free ethyl ether and the four subsequent extraetions with 25- to 30-ml, portions. The last extraetions should be almost colorless. The ether extraet is washed five times with 25-ml. portions of water, after which it is dried over 20 grams of anhydrous sodium sulfate for 1 hour at room temperature. The ether extraet is evaporated to approximately 15 ml. under reduced pressure in a 50° C. water bath and then transferred to a 25-ml. volumetric flask with dry petroleum ether. If the solution still contains some moisture, as indicated by eloudiness, it should be dried with a small amount of sodium sulfate.

In the chromatographie separation 10 ml. of this solution are adsorbed on a column (20 × 135 mm.) of 3 parts of calcium hydroxide (Braun's Lot No. 10,588) and 2 parts of Hyflo Super-Cel. The ehromatogram is developed with a mixture of 60% benzene (thiophene-frec) and 40% dry petrolcum ether. The two lowest bands containing β-carotene and cryptoxanthol are eluted separately. The vitamin A fraction is collected until the luteol begins to give a yellow color to the eluate. If the column is properly paeked, the β-carotene and cryptoxanthol bands are easily distinguished. The luteol band should be sharp as it nears the bottom of the column. Fifty to 80 I.U. of vitamin A and 150 to 200 micrograms of total carotenoids (as β-carotene) can be handled satisfactorily with the column described. A total volume of 250 to 500 ml. of combined β-carotene, cryptoxanthol, and vitamin A eluate is the optimum range for best results.

After removing the solvent from the β-carotene and cryptoxanthol fractions, they are taken up in 10 ml. of petroleum ether and the light transmission is measured by means of the Evelyn photoelectric colorimeter with the standard 440 mμ filter. These solutions are then recombined with the vitamin A fraction and the solution is evaporated under redueed pressure at 50° C. to approximately 15 ml. This solution is then transferred to a 25-ml. volumetric flask with redistilled ehloroform. Chloroform is used in this transfer beeause it is a somewhat better solvent than petroleum ether. Ten milliliters of this solution are evaporated to dryness in a colorimeter tube and redissolved in 2 ml. of ehloroform. The tube is placed in the Evelyn instrument, 8 ml. of antimony trichloride reagent are added, and the light transmission is measured with the standard 620 filter. The blue color should be read within 5 to 10 seconds after the antimony trichloride reagent is added. This color should be brilliant and clear. Cloudiness in the solution at this point usually indieates insufficient care in excluding adsorbent or moisturc.

One gram of U.S.P. referenee eod liver oil No. 2 was saponified and extraeted by the above procedure in establishing the calibration eurve for the vitamin A. A sample of β-carotene, purified by chromatography, was used as the standard for the determination of β-carotene, eryptoxanthol, and total carotenoids.

RESULTS AND DISCUSSION

Results obtained on dried egg samples with this method were reproducible and showed good agreement with those obtained by bioassay (Table III). Eight samples of dried eggs of different storage history were tested for vitamin A by both methods with a maximum deviation of 10% in results. Eight replicate chemical determinations on a single sample showed similar reproducibility.

Several precautions were found necessary with respect to materials and procedure; other precautions were taken without testing their importance. One of the most important precautions to be observed in using this method is the preparation of the petroleum ether. Some samples of commercial petroleum ether contain an impurity which produces a green color when mixed with the antimony trichloride reagent. The use of sueh petroleum ether without purification results in erratic vitamin A values. The petroleum ether was purified as follows:

Four to five gallons of commercial petroleum ether (boiling point 65° to 67° C.) were percolated through a column (7 × 35 cm.) of silica gel (The Davison Chemieal Corporation, No. 659,-528-2000). The percolate was then placed in a 5-gallon flask and stirred meehanically for several hours with each of two successive portions of concentrated sulfuric aeid. The two phases were separated and the last trace of aeid was removed by treatment with a dilute solution of sodium hydroxide. The petroleum ether was then stirred for a few hours with alkaline potassium permanganate, after which it was drawn off, distilled, and dried over anhydrous sodium sulfate.

Trouble was encountered with the adsorbent when the humidity in the laboratory became high. Under these conditions the carotenoids moved too rapidly down the column and it was impossible to separate the β-carotene and cryptoxanthol fractions. This difficulty was avoided by storing the adsorbent in a desiccator over sulfuric acid and removing it only long enough to pack the column.

Among the necessary operations was the addition of the β-carotene and eryptoxanthol fractions back to the vitamin A

Table II. Recovery of Vitamin A from a Calcium Hydroxide Chromatogram

Source of Vitamin A	Vitamin A Adsorbed	Vitamin A Eluted	Recovery
	I.U.	*I.U.*	%
U.S.P. reference cod liver oil No. 2	24	26	108
	122	115	95
	235	243	103
Crystalline vitamin A alcohol[a]	19.7	19.0	96
	20.3	18.5	91

[a] Purchased from Distillation Products, Inc.

Table III. Comparison of Chemical and Bioassay Methods[a]

	Chemical Methods							
	Not Chromatographed		Chromatographed				Bio-assay, Vita-min A	I.U. (Chemical) / I.U.(Bioassay) × 100
Sample	Total carote-noids[b]	Vitamin A[c]	β-Carotene	Crypto-xanthol[b]	Vitamin A	Total vitamin A activity[d]		
	Micro-grams/ gram	*I.U./gram*	*Micrograms/gram*		*I.U./gram*		*I.U./gram*	
3B	100.6	71.4	3.0	3.2	39.0	46.7	44.0	106
4B	106.9	93.0	3.4	3.6	31.5	40.2	41.0	98
5B	102.0	75.5	4.3	4.8	30.7	41.9	43.0	98
8B	70.3	54.7	2.6	3.0	21.2	28.0	30.0	94
9B	114.0	82.0	3.3	3.3	30.2	38.5	41.0	94
10B	101.3	74.0	2.9	3.5	25.5	33.3	36.0	93
12B	79.5	65.0	2.5	2.7	26.0	32.4	32.0	101
13B	72.9	..	2.2	3.6	20.9	27.6	25.0	110
14	98.2	80.0	2.8[e]	3.4[e]	35.1	42.6	45.0	95
	94.5	..	..	..	37.0	44.5	..	99
	95.0	..	..	..	36.4	43.9	..	97
	97.6	..	..	..	36.1	43.6	..	97
	91.9	..	..	..	33.9	41.4	..	92
	97.7	..	..	..	36.8	44.3	..	98
	..	..	..	..	36.9	44.4	..	99
	95.7	..	..	..	33.4	40.9	..	91

[a] All values expressed on moisture-free basis.
[b] Expressed as β-carotene.
[c] Calculated from blue color given by total unsaponifiable extract.
[d] I.U. of vitamin A equivalent to 0.6 microgram of β-carotene and 1.2 micrograms of cryptoxanthol (*6*).
[e] Four determinations gave 2.8 and 3.4 as average values for β-carotene and cryptoxanthol, respectively, on this sample.

eluate before reacting with antimony trichloride, since it was found that small amounts of the vitamin were contained in these two eluates. This overlapping of vitamin A with the carotene and cryptoxanthol bands was not prevented by complete removal of the ether from the petroleum ether–ether solution of unsaponifiables before chromatographing. However, the bands were sharper when pure petroleum ether was used. β-Carotene and cryptoxanthol were shown experimentally to give only 7 and 11%, respectively, of the blue color given by vitamin A on reaction with antimony trichloride. Therefore, the effects of the β-carotene and cryptoxanthol on the total blue color development were so small as to be negligible. With one lot of eggs which was analyzed an additional small band was observed in the region of the β-carotene and cryptoxanthol bands. Depending upon the dietary constituents of the hen, still other bands might be expected. Should these occur in substantial amounts a correction would be necessary.

The following precautions were used without testing their importance.

Freshly distilled ethyl ether was used for extraction of the vitamin. Anhydrous ethyl ether (alcohol-free) was allowed to stand several days over potassium hydroxide and then redistilled. Chloroform (A.R.) was distilled over anhydrous potassium carbonate and stored in a brown bottle. The antimony trichloride reagent was made up by dissolving 22.5 grams of antimony trichloride in 100 ml. of redistilled chloroform, filtering, and storing in a brown bottle. A pipet calibrated to deliver 8 ml. and equipped with a rubber bulb for rapid delivery was used for adding the antimony trichloride reagent directly to the sample in the colorimeter tube. An all-glass distillation apparatus was used for the removal of solvent.

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Measuring Coverage and Film Thickness of Printing Ink and Paint Films

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THE determination of the amount of paint, lacquer, or printing ink on a given area of a certain surface—i.e., paper, metal, wood, or plastic—is of considerable interest in various technical fields. In the printing process, for example, it is desirable to print as thin a film as possible because this reduces, or eliminates completely, technical difficulties such as slow drying, offset, and fill-up in the half-tone plates, which are frequently encountered when it is necessary to use a greater film thickness. Similar considerations apply in the field of paint technology. Furthermore, the optical properties of a paint or printing ink film are closely related to the film thickness. Both the reflection and transmission spectra are functions of the film thickness. Hiding power depends essentially on the over-all absorption coefficient as a function of the wave length and the thickness of the particular specimen. The relationship between coverage and the actual structure to which the film is applied is another important problem in the paint and printing ink field.

Several methods have been described in the literature for measuring the coverage or the thickness of such films. One of the simplest procedures consists in weighing the specimen before and after application of the film-forming material. When applied to printing on paper this method is not satisfactory because an accurate weight determination (within 0.3 mg.) of a sheet of paper is impossible. This difficulty has been overcome (*2*) by using as a printing plate a very thin sheet of copper (or some other suitable metal) which is held firmly to a heavy base during the printing operation. The weight of the copper sheet before and after printing can easily be determined to within ±0.1 mg. Although the method in this form is very accurate, it has several drawbacks which limit its usefulness considerably. The film thickness can be determined only if the material is applied as described above; it is not satisfactory if a film-forming material containing some volatile matter is employed. An electrical method (*3*), based on capacity measurements, cannot be applied for measurements upon paper surfaces. Optical methods (*1*, *4*), based on interference patterns or on microscopic examination of microtome sections, are either not applicable to paper surfaces or too impractical to be useful.

Two methods developed in this laboratory overcome most of the difficulties mentioned above. Both methods make use of a tracer material which is added to the film-forming material. In one case the tracer material is a dyestuff, in the other a radioactive isotope. Although both methods were developed primarily to study the film thickness of printing inks, they are believed to be applicable to paints, lacquers, or other protective coatings.

METHODS

Dyestuff as Tracer Material. A known amount of dyestuff is added to a printing ink, and prints made from this ink are put into a Soxhlet extractor. Using alcohol, for example, it is possible to remove the dyestuff quantitatively from the print. The amount of dyestuff present in the alcohol solution is determined by measuring the transmission coefficient at a given wave length, following the standard procedure of quantitative colorimetric analysis.

To obtain satisfactory results the following factors should be carefully considered. The absorption spectra of the extraction and of the standard solutions (which furnish the calibration curve) must be identical, as shown in Figure 1, at least in the wave-length region where the colorimetric measurements are made. The presence of oils and resins in the extraction may in certain cases shift the absorption bands sufficiently to produce erroneous results. The extraction must be complete. This can easily be verified by repeated extractions or by a comparison of results obtained with the gravimetric method (*2*) and the dyestuff method, using the same prints in both cases. Data given in Table I, which are typical of a large number of printing inks, show that the extraction is complete. The absorption can also be disturbed by the presence of colloidal particles, such as carbon black pigments, which might produce a certain amount of turbidity. This point can easily be checked by running extractions

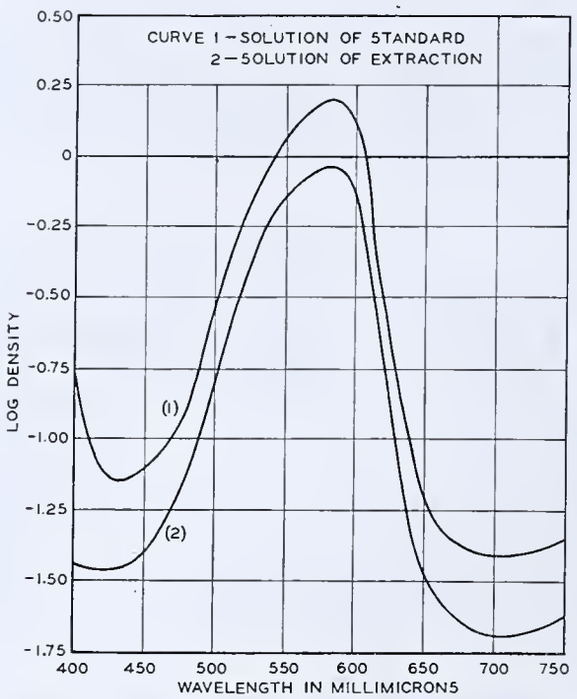

Figure 1. Log *D* Curves of Two Dyestuff Solutions

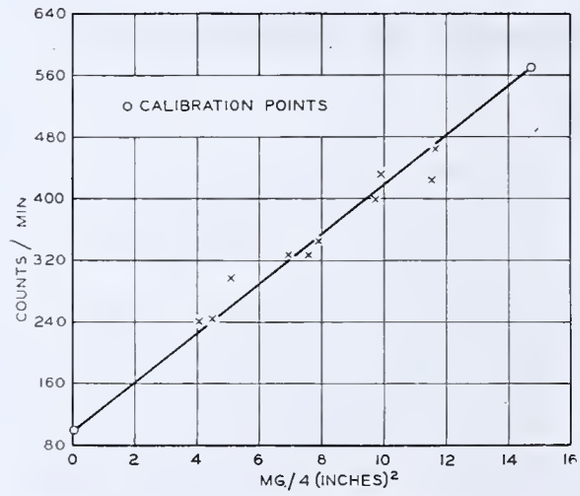

Figure 2. Counting Rate as a Function of Amount of Ink on a Given Print Area

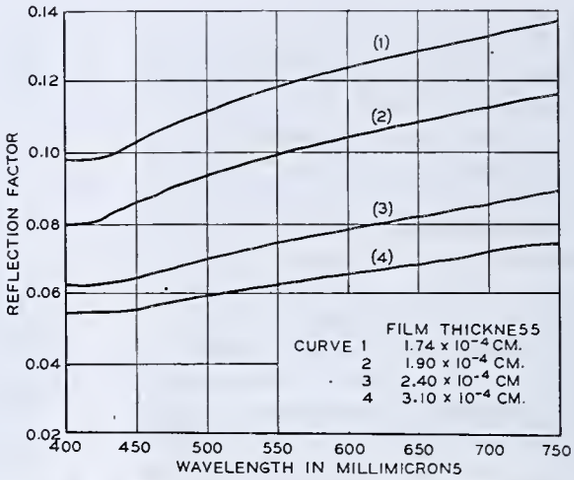

Figure 3. Reflection Spectrum of Prints of Different Film Thickness

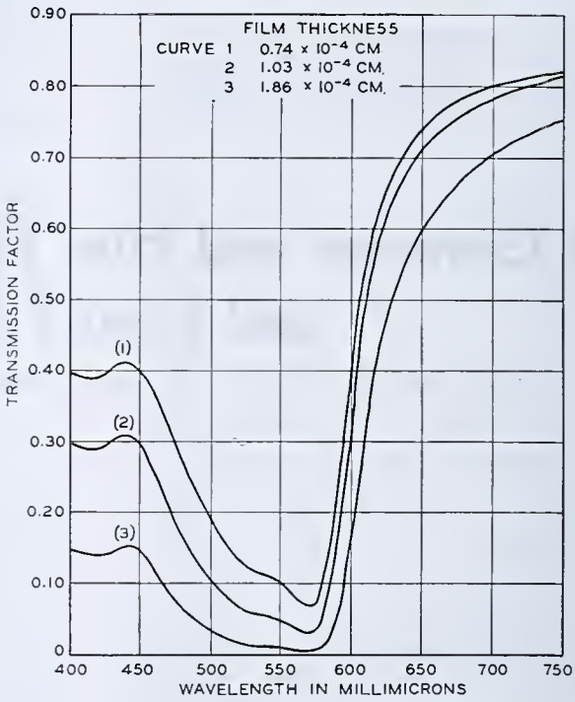

Figure 4. Transmission Spectrum of Prints of Different Thickness

on a printing ink containing no dyestuff. In this case the absorption spectrum of the extraction should be the same as, or at least very close to, the absorption spectrum of the solvent. Finally, it must be ascertained that the absorption spectrum of the dyestuff does not change irreversibly at the temperature at which the extraction is carried out. When all these factors are taken into account the results obtained by this method are judged to be accurate to at least ±7%.

Radioactive Isotope as Tracer Material. A known quantity of a radioactive compound (preferably a liquid) is added to a printing ink. Using the modified weighing method described above, a print is made with a known amount of ink on a given area of paper. The activity of this print is measured with a conventional Geiger counter set. (The instrument used in these experiments is built by the Cyclotron Specialties Corporation, Moraga, Calif.) This quantity and the background activity due to cosmic radiation give the calibration curve (Figure 2). The calibration curve changes with time because of the constant decay of the radioactive isotope.

In order to obtain satisfactory results the following points should be considered. The position of the counter tube relative to the sample must remain the same because the number of radioactive disintegrations picked up by the counter tube depends among other things, on the geometry of the experimental setup. The radioactive material should be uniformly distributed throughout the printing ink. This can best be achieved by using a material which is miscible with the liquid phase of the printing ink. Finally, the activity of the specimen under test should be at least twice that due to the background radiation. An accuracy of ±3% can easily be obtained with this method.

Table I. Amount of Printing Ink on a Given Print Area

(Dyestuff used, methyl violet. Extraction solvent, alcohol. Extraction time, approximately 30 minutes per print)

Gravimetric Method	Dyestuff Method
Gram/25 sq. inches	
0.0521	0.0480
0.0283	0.0320
0.0331	0.0314
0.0310	0.0314
0.0430	0.0438
0.0380	0.0374

Table II. Amount of Ink on a Given Area of a Print for Different Papers

Machine Coated Paper	Amount of Ink
	Gram/sq. cm.
1	0.405×10
2	1.03×10^{-4}
3	1.21×10^{-4}
4	1.40×10^{-4}
5	2.5×10^{-4}

RESULTS

Table I gives comparative data obtained with the dyestuff and the gravimetric methods for a series of prints, using the same ink and paper. Table II shows the amount of ink taken up by various papers, keeping all other variables constant. The dyestuff method was used to obtain these data.

Figures 2, 3, and 4 show results using a radioactive isotope as tracer material. A radioactive isotope of phosphorus in the form of phosphorus pentachloride (obtained from the Radiation Laboratories, Massachusetts Institute of Technology) was used in these experiments. Figure 2 shows a comparison between the radioactive and the gravimetric methods. The scattering of some of the points on this graph is due to the nonuniform distribution

of the ink on the paper surface. For the gravimetric method the amount of ink per unit area was determined from a print area of 12.5 × 12.5 cm. (5 × 5 inches); the radioactive measurements, however, were made on a print area of 5 × 5 cm. (2 × 2 inches). Figure 3 shows the change of the reflection coefficient (measured with a GE recording spectrophotometer) as a function of the film thickness; Figure 4 shows the change of the transmission spectrum (between 400 and 700 millimicrons) of a printing ink printed on cellophane paper as a function of the film thickness.

There are limitations to both methods. Although the dyestuff method can be applied in many cases where the gravimetric method breaks down, it is still a relatively slow procedure. Furthermore, in many cases it might be undesirable or even impossible to incorporate a dyestuff into the material under consideration. The most important limitation with regard to the radioactive tracer method is that imposed by the radioactive decay of the isotope. The particular isotope in this work has a half-life of 15 days. Using a fairly strong preparation (60 microcuries per cc. of varnish) the coverage on an area of 25 sq. cm. (4 square inches) can be determined for a period of about 6 weeks. On the other hand, the advantages gained by the use of either of the methods outweigh the limitations. Both methods are accurate and can be performed simply and more universally than existing methods.

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Spectrophotometric Procedure for Quantitative Estimation of Vitamins D

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A modified antimony trichloride reagent using ethylene chloride as a solvent is suggested for the estimation of vitamin D. This modified reagent is readily reproducible, is stable over several weeks, and reacts with the vitamin to give a salmon-pink solution exhibiting an absorption maximum at 500 mμ. The reagent has been applied to the estimation of vitamin D in a variety of materials. A simple chromatographic procedure suggested for the separation of vitamin D from vitamin A and sterols utilizes a weak source of ultraviolet rays to permit observation of the materials on the column. The results of the chemical procedure are in good agreement with those obtained by biological assays.

A NUMBER of independent physicochemical procedures have been proposed for the determination of the vitamin D content of fish liver oils and other vitamin preparations. Some of these procedures have given good results with highly purified samples of vitamins D_2 and D_3, but their successful application to the assay of common fish liver oils has been limited.

Among the purely physical procedures is that of Reerink and van Wijk (*13*), in which the concentration of vitamin D in a solution of irradiated ergosterol is determined by measuring the optical density at the absorption maximum of 265 mμ. Töpelmann and Schuhknecht (*18*), Fuchs and Beck (*6*), and Ol Khin (*12*) made use of this procedure, and Marcussen (*9*) applied the technique to a few fish liver oils.

Various colorimetric methods have received more extensive study and application. Shear (*16*) suggested a colorimetric method based upon the reaction between vitamins D and aniline–hydrochloric acid reagent. Stoeltzner (*17*) treated the vitamin with phosphorus pentachloride, but Christensen (*3*) showed that this reaction was not specific for vitamin D. Cruz-Coke (*4*) based a test on the development of a green color when an alcoholic solution of irradiated ergosterol was treated with hydrochloric acid. Meesemaecker (*10*) showed that irradiated ergosterol gave a yellow-green solution when treated with a modified Liebermann reagent. Robinson (*15*) suggested a colorimetric method based on the yellow color formed when an alcoholic solution of vitamin D was boiled with sodium nitrite and acetic acid.

A more quantitative method was proposed in 1936 by Halden and Tzoni (*7*, *8*, *19*), who showed that a deep violet color is obtained when an alcoholic solution of vitamin D is treated with anhydrous aluminum chloride in the presence of pyrogallol. Cholesterol and ergosterol do not interfere with the test, but vitamin A and the carotenoids must be quantitatively removed. The authors report that the method is sensitive to 2 micrograms of the pure vitamin.

Perhaps the most widely employed test is that employing a solution of antimony trichloride in chloroform, which was proposed by Brockmann and Chen (*2*) in 1936. Brockmann (*1*) used this method in his work on the isolation and identification of vitamin D from tuna liver oil, but it is applicable only to concentrated solutions of vitamin D which are relatively free from inactive sterols, vitamin A, and the inactive irradiation products from ergosterol. Wolff (*22*) asserted that the error of the procedure is ±19%, and Ritsert (*14*) reported that the method was inapplicable to fish liver oils or to mixed irradiation products from the sterols.

Nield, Russell, and Zimmerli (*11*) modified the Brockmann-Chen reagent by adding a small amount of acetyl chloride, and obtained a marked increase in stability, reproducibility, and sensitivity of reaction. Ewing, Kingsley, Brown, and Emmett (*5*) applied this modified reagent to the assay of fish liver oils, using a chromatographic procedure to remove interfering sterols and vitamin A.

This review indicates the nature of the obstacles to be overcome in the application of physical or chemical methods to the determination of vitamin D. Vitamin A, sterols, and carotenoids interfere with the direct spectrophotometric and colorimetric methods, and must be removed quantitatively. Some of the proposed reagents lack specificity and sensitivity, and are difficult to prepare or to reproduce. In many cases, the reaction between vitamin D and the reagent gives a fleeting color, and the determination must be completed within a few seconds after the solutions are mixed.

In order to overcome some of these difficulties, the authors have employed a simple chromatographic technique for separation of the vitamin from interfering substances, and have devised a modified antimony trichloride reagent which offers increased ease of preparation, increased reproducibility, and greater sensitivity and produces a more permanent color with vitamin D. The procedure has been applied to the assay of a number of fish liver oils, irradiated materials, and multivitamin preparations. Most of the results have been checked by biological assays.

EXPERIMENTAL

Preparation of Reagent. Care must be taken to ensure that all materials are free from moisture. Twenty grams of reagent grade antimony trichloride are mixed with approximately 10 grams of anhydrous calcium chloride, and pulverized in a mortar. The powdered material is shaken with 100 ml. of ethylene chloride (boiling point 83–84° C.) and filtered rapidly through anhydrous sodium sulfate. Two milliliters of acetyl chloride (boiling point 49–52°) are added to each 100 ml. of filtrate. This reagent is ready for use after 30 minutes, and is stable over a period of at least 10 weeks.

Table I. Extinction Coefficients of Products of Reaction with Vitamin D

(Each reagent contained 20 grams of $SbCl_3$ per 100 ml. of solvent and 2 ml. of acetyl chloride per 100 ml. of reagent)

Reagent No.	Solvent	Extinction Coefficient	Remarks
1	Chloroform	1800	Nield's reagent
2	Methylene chloride	2070	Reagent unstable
3	Ethylene chloride	2200	....
4	Propylene chloride	2100	Less stable than 3
5	Carbon tetrachloride	..	No reaction
6	Monochlorobenzene	1400	....

Table II. Relationship between Absorption at 500 mμ and Vitamin Concentration

Vitamin Concentration γ/10 ml.	Density
5	0.15
10	0.26
15	0.35
20	0.445
25	0.56
30	0.65
35	0.74
40	0.85
50	1.05

Preparation of Adsorption Column. An Allihn sugar tube, 2 × 10 cm., fitted with a fritted filter of medium porosity may be used for the preparation of the adsorption column. The tube is fitted to a suitable suction flask, vacuum is applied, and small portions of a 1 to 1 mixture of magnesia (Baker's Reagent Grade is suitable) and diatomaceous earth (Celite) are packed firmly until a column approximately 6 cm. in length is obtained. A 1-cm. layer of anhydrous sodium sulfate is placed on the top of the column, and packed tightly.

Saponification and Extraction. A quantity of fish liver oil sufficient to yield approximately 25 micrograms or more of the vitamin is boiled 30 minutes with freshly prepared 12% alcoholic potassium hydroxide, maintaining a ratio of 2.5 grams of potassium hydroxide per gram of oil. The alcohol volume is maintained constant throughout. At the end of the saponification period, the mixture is cooled, diluted with an equal volume of distilled water, and extracted 10 times with 50-ml. portions of anesthesia grade ethyl ether. (In case the sample weighs 10 grams or more, the extraction technique may be modified as follows: After addition of the water, add 25 ml. of ethyl acetate and 50 ml. of petroleum ether, and shake 30 seconds. Allow to stand 2 minutes, transfer the aqueous layer to another separatory funnel, and extract 10 times with a mixture of 10% ethyl ether and 90% petroleum ether. Combine extracts and continue as above.)

The extracts are combined and washed with distilled water until the washings are neutral to phenolphthalein. The extracts are dried by filtration through anhydrous sodium sulfate, the ethyl ether is removed by evaporation, and the residue is dissolved in a minimal volume of petroleum ether for chromatographing.

In the case of capsules, either vitamin D or multivitamin preparations, 5 or more capsules may be opened, and the oily contents quantitatively removed by repeated washing with ethyl ether. The ether is removed by evaporation, and the oily residue saponified as above. In an alternative procedure, which has proved successful with solid-type gelatin capsules, the capsules are boiled with the alcoholic potassium hydroxide exactly as in the case of the oils.

Tablets and other solid materials are pulverized in a mortar, and the entire mass is boiled with alcoholic potassium hydroxide for 30 minutes. The solids are removed by centrifugation, reheated with fresh portions of alcoholic potassium hydroxide, and again centrifuged. The liquids are combined and extracted with ethyl ether as in the case of the oils. The solid portion is transferred to a Büchner funnel and repeatedly extracted with ethyl ether, and the extracts are combined with those from the liquid portions.

Chromatographic Technique. The column is wetted with 50 ml. of petroleum ether, the ethereal portion from the saponification procedure is added, and the chromatogram is developed with 5-ml. portions of petroleum ether. The procedure is observed under ultraviolet rays of low intensity from a lamp described by Wilkie and De Witt (*21*). Several distinct zones of fluorescence may be observed, starting from the top of the column:

1. A narrow band of intense pale blue fluorescence.
2. A broad band of greenish-yellow fluorescence which contains vitamin A.
3. A narrow band of light gray fluorescence.
4. Two narrow bands approximately 2 mm. apart showing a bluish fluorescence.
5. A narrow band of gray-blue fluorescence ("sterol fraction").

Continued washing of the column with petroleum ether elutes zones 3, 4, and 5. The eluate from each zone is collected separately and evaporated on the steam bath. The residues are transferred to suitable glass-stoppered volumetric flasks, and made to volume with petroleum ether. Aliquots from each portion are examined by the spectrophotometric procedure to determine that separation of vitamin D from other materials is complete. In case of incomplete separation, a second chromatographing may be necessary.

The eluate from zone 5 contains the bulk of the inactive sterols that from zone 4 contains vitamin D, and that from zone 3 may contain small amounts of vitamins A and D. All eluates are made to the same volume, which should be adjusted so that the portion from zone 4 contains approximately 1000 I.U. of vitamin D per milliliter.

Spectrophotometric Procedure. The petroleum ether solution (0.5 ml.) is placed in a 10-ml. glass-stoppered graduated cylinder and 9.5 ml. of the antimony trichloride reagent are added. The cylinder is shaken vigorously, the solution transferred to the absorption cell, and the $E^{1\%}_{1\ cm.}$ at 500 mμ determined on the Beckman spectrophotometer. Readings are made at the end of 30 seconds, and at 1-minute intervals for the first 5 minutes after starting to add the reagent to the vitamin sample. Under these conditions, sterols react slowly to give a low initial optical density which increases very rapidly to reach a maximum after approximately 5 minutes. Vitamin D reacts rapidly, giving a maximum optical density within 1 minute, and remaining constant for 5 to 10 minutes.

The potency of the vitamin preparation is obtained by multiplying the $E^{1\%}_{1\ cm.}$ of the solution by the factor of 18,200, obtained by repeated checks of the reagent against crystalline vitamin D_2 and D_3.

DISCUSSION OF PROCEDURE AND RESULTS

The preliminary experiments in this study dealt with the reagents that had been proposed, and with possible modifications designed to increase the accuracy or ease of determination. The reagent proposed by Nield, Russell, and Zimmerli (*11*) was found relatively satisfactory, but it possessed several objectionable features. The chloroform used as the solvent had to be freed of all traces of alcohol and moisture, and the purified solvent was unstable. The reagent was hygroscopic, and showed an increased sensitivity toward sterols if allowed to stand several weeks.

Further experiments were designed to study the effects of changes in the concentration and identity of each of the three components of the reagent. It was found that the concentration of antimony trichloride and of acetyl chloride could be varied over wide limits without producing significant changes in the sensitivity of the reagent, and that the substitution of mono-, di- and trichloroacetyl chlorides for acetyl chloride produced little change. However, a marked change in the reaction was produced when chloroform was replaced by other halogenated solvents, as is shown in Table I. The use of methylene chloride, ethylene chloride, or propylene chloride gave a reagent of increased sensitivity, as is shown by the increase in $E^{1\%}_{1\ cm.}$ values. However, the methylene chloride reagent was unstable, and decomposed within a few hours after preparation. The reagent prepared from ethylene chloride was slightly more sensitive and was stable over a period of at least 10 weeks. The salmon-pink color of the solution resulting from the reaction of this reagent with vitamins D_2 and D_3 shows maximum absorption within 30 seconds, and remains stable for several minutes. The solution exhibits a narrow absorption band with maximum absorption at 500 mμ, and absorbs none of the incident light at 550 mμ. A

shown in Table II, the amount of absorption is proportional to the concentration of vitamin D, and concentrations of the vitamin as low as 5 micrograms per 10 ml. of solution may be measured under the experimental conditions.

This initial phase of the work involved studies of the reaction between crystalline vitamins D_2 and D_3 and the reagent. The second phase involved the application of the reagent to the estimation of vitamin D in a variety of materials. Crystalline vitamins D_2 and D_3 were used as standards of reference in this study. The results of the spectrophotometric determination were checked by biological assays.

The chromatographic technique is unique in that it employs a simple procedure permitting rapid separation of vitamin D from interfering sterols and vitamin A. The use of the weak source of ultraviolet light permits close observation of the column, and enables the analyst to locate the different substances with greater ease and accuracy than is possible by the use of dyes as in the method of Brockmann (*1*).

The experimental results obtained by the application of the method to a limited number of samples are given in Table III, and are self-explanatory. In general, the results are in good agreement with those obtained by biological assay. Differences, where they occur, may be due to several causes, including the errors inherent in all biological assays. Another possible cause of discrepancy arises from the fact that the bioassays were designed to show that the potency of the material was approximately equal to or greater than the given figure, and would not permit close estimation of the exact potency.

Table III. Results of Spectrophotometric and Biological Assays of Vitamin D Carriers

Sample No.	Vitamin D Content: Bioassay	Vitamin D Content: Spectrophotometric	Remarks
	U.S.P. units per gram		
	A. Vitamins D_2 and D_3 in Oil		
72442	200,000	198,000	D_3 in corn oil. A.O.A.C. assay
12313	400,000	422,000	Irradiated ergosterol
12314	400,000	389,000	Irradiated ergosterol
34074	10,000	8,950	D_2 in sesame oil
73142	166,500	165,000	D_3 in corn oil
B 72144	200,000	198,300	No bioassay, figure from label declaration
	U.S.P. units per capsule		
74290	50,000	57,300	D_2 in capsules
60910	50,000	48,900	Capsules
VA 9108	50,000	52,650	Capsules, with semisolid gelatin medium
60932	50,000	54,550	Solid-type gelatin capsules
59801	50,000	53,000	No bioassay, figure from label
58347	50,000	57,950	No bioassay, figure from label
VA 5573	40,000 approx.	42,800	Label declares 50,000 per capsule
51408	50,000	47,800	
	B. Vitamins A and D in Oil		
	U.S.P. units per gram		
92244	115	113	
23606	400	395	
12073	....	160	More than 85 U.S.P. units per gram by bioassay
23607	400	410	
72144	10,000	10,385	50,000 A per gram
61544	....	42	Less than 50 units per gram by bioassay
	U.S.P. units per capsule		
11143	1,000	1,040	5000 A per capsule
2243	1,000	1,020	5000 A per capsule
41543	1,000	1,050	5000 A per capsule
	U.S.P. units per gram		
VA 7048	10,000	11,250	50,000 A per gram
10143	10,000	9,725	50,000 A per gram
	U.S.P. units per capsule		
1144	1,000	950	5000 A per capsule
	C. Multivitamin Preparations		
92044	1,000	1,050	6 vitamin capsules
91944	200	205	Hexavitamin capsules
	U.S.P. units per tablet		
C 1234	200	220	Hexavitamin tablets

Table IV. Results of Spectrophotometric and Biological Assays of Vitamin D Carriers

Sample No.	Vitamin D Content: Bioassay *A.O.A.C. units/g.*	Vitamin D Content: Spectrophotometric $\gamma/g.$	Vitamin D Content: Spectrophotometric *A.O.A.C. units/g.*[a]
1	1,000,000	20,125	986,000
2	500,000	10,100	494,000
3	1,150,000	22,300	1,092,700
4	575,000	10,800	529,000

[a] Calculated on basis that 1 gram of crystalline D_3 contains 49,200,000 A.O.A.C. units.

The limitations of the method have not been fully determined. With high-potency materials such as those listed in the first part of Table III, the spectrophotometric procedure has shown a high degree of accuracy and reproducibility.

Several commercial samples were examined by the spectrophotometric procedure, and were then assayed biologically in the laboratories of the U. S. Food and Drug Administration. Portions of these samples were resubmitted for spectrophotometric examination at a later date without knowledge that they had been previously analyzed. The results obtained in the second examination were within $\pm 2\%$ of the previous values.

A further check of the procedure involved the assay of a series of oils prepared by J. Waddell of E. I. du Pont de Nemours & Co. The samples were submitted as unknowns, with no declaration of potency. The results of this study are given in Table IV, in which the spectrophotometric values are compared with the potencies assigned by Waddell after biological assays in three independent laboratories. The biological values are expressed in terms of A.O.A.C. units, while the spectrophotometric values are in terms of micrograms. Since Waddell (*20*) reports that crystalline vitamin D_3 contains 49,222,000 A.O.A.C. units per gram, the agreement between the spectrophotometric and biological values is fairly close.

Although the values shown in part B of Table III indicate that it is possible to obtain satisfactory results in the spectrophotometric examination of low-potency oils, it is felt that the question of the applicability of the procedure to such materials has not been fully determined. The reproducibility of results in duplicate assays is less satisfactory than in the case of high-potency materials, and some modifications or refinements of procedure may be necessary in order to obtain uniformly satisfactory results.

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Precipitation of Platinum Metals by Organic Monosulfides

Determination of Platinum with Thiophenol and Rhodium with Thiobarbituric Acid

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This report deals with a preliminary investigation of organic monosulfide compounds as analytical reagents for the platinum metals. The action of seven organic monosulfide compounds with some of the platinum and related metals is described. It has been found that rhodium may be determined gravimetrically with thiobarbituric acid. Thiophenol may be used as a precipitant in the gravimetric determination of platinum.

THE reactions of organic sulfide compounds with many metals have been studied extensively. Zeise (*14*) in 1834 chose the name "mercaptan" for C_2H_5SH because of the reaction of this compound with mercury. He also described (*15*) its reaction with platinum. Many references have been made in the literature to further researches on organic sulfide compounds with platinum metals, most of them concerned with the study of the methods of preparation and stereoisomerism of the various compounds produced. Some of these reactions have formed the basis for analytical procedures for platinum metals. Berg and coworkers (*4–8*) investigated the action of thionalide (thioglycollic-β-aminonaphthalide) with many metals, and Kienitz and Rombock (*10*) used it for the volumetric estimation of rhodium. Rogers, Beamish, and Russell (*12*) determined ruthenium gravimetrically by precipitation with thionalide.

The reactions of organic monosulfide compounds with platinum metals have been examined with a view to finding useful analytical methods for their determination and relating the configuration of the organic compounds with their ability to precipitate these metals. The precipitation of several platinum and associated metals under various conditions of acidity with seven organic monosulfide compounds has been investigated. In some cases the precipitation of various platinum metals is complete. Rhodium may be determined gravimetrically with thiobarbituric acid. Thiophenol may be used as a precipitant in the determination of platinum.

PRECIPITATION REACTIONS

Standard solutions of platinum, palladium, rhodium, iridium, gold, nickel, copper, and iron containing approximately 1 mg. of metal per ml. of solution were prepared by dissolving the pure metal or a suitable pure salt in aqua regia, hydrochloric acid, or water, removing the nitric acid, if present, by fourfold evaporation to dryness in the presence of hydrochloric acid, and finally dissolving in a solution containing 1 ml. of concentrated hydrochloric acid per liter.

A 10-ml. aliquot of the standard metal solution under investigation was diluted and acidified to one of the six acid conditions given in Table I. The organic reagent was added and the solution heated and maintained at boiling for 2 hours. The total volume of the solution was maintained at 200 ml. by additions

Table I. Precipitation of Platinum Metals

N. No precipitation, or precipitate contained none of metal under investigation
S. Very slight precipitation
P. Partial precipitation
C. Complete precipitation; filtrate contained less than 0.02 mg. of metal under investigation

			Acid Strength					
Reagent	Amount of Reagent	Metal, 10 Mg.	1:3 aqua regia 0.012 *N*	HCl 0.012 *N*	HNO_3 0.012 *N*	1:3 aqua regia 0.60 *N*	HCl 0.60 *N*	HNO_3 0.60 *N*
Allyl thiourea	1 ml. of 2% w/v soln. in 95% ethanol	Pt	N	N	N	N	N	P
		Pd	P	C	C	N	P	P
		Rh	N	N	N	N	P	P
		Ir	N	N	N	N	N	N
		Au	P	C	P	N	C	C
		Ni	N	N	N	N	N	N
		Cu	N	N	N	N	N	N
		Fe	N	N	N	N	N	N
Phenyl thiourea	1 ml. of 3% w/v soln. in 95% ethanol	Pt	P	P	P	N	P	P
		Pd	C	C	C	P	P	P
		Rh	P	P	P	N	P	S
		Ir	S	N	S	N	P	N
		Au	P	C	P	N	P	P
		Ni	N	N	N	N	N	N
		Cu	S	S	S	N	S	N
		Fe	N	N	N	N	N	N
s-Di-*o*-tolyl thiourea	10 ml. of 0.8% w/v soln. in 95% ethanol	Pt	P	P	P	P[a]	C	P
		Pd	P	P	C	P	C	C
		Rh	P	P	P	P	C	P
		Ir	N	N	N	N	N	N
		Au	P	P	P	P	C	P
		Ni	N	N	N	N	N	N
		Cu	P	P	P	P	P	P
		Fe	N	N	N	N	N	N
Thiophenol	1 ml. of 4% v/v soln. in 95% ethanol	Pt	P	C	C	P	P	P
		Pd	C	C	C	P	C	C
		Rh	P	P	P	P	P	P
		Ir	P	P	P	P	P	P
		Au	C	C	C	N	C	C
		Ni	N	N	N	N	N	N
		Cu	P	P	P	N	S	P
		Fe	N[b]	N[b]	N[b]	N	N[b]	N
Phenyl thiohydantoic acid	2 ml. of 2.1% w/v soln. in 95% ethanol	Pt	P	P	P[a]	N	P	P
		Pd	P	P	C[c]	P	P	C[c]
		Rh	P	P[a]	P	P	P	P
		Ir	S	S	S	N	N	N
		Au	C	P	C	N	C	C
		Ni	N	N	N	N	N	N
		Cu	P	P	P	P	P	P
		Fe	N	N	N	N	N	N
Thiobarbituric acid	2.5 ml. of 1.4% w/v soln. in 95% ethanol	Pt	C	C	C	C	C	P
		Pd	C	C	C	C	C	P
		Rh	P	C	C	P	P	P
		Ir	P	C	P	P	P	N
		Au	C	C	C	P	C	N
		Ni	N	N	N	N	N	N
		Cu	S	S	S	N	N	N
		Fe	N	N	N	N	N	N
s-Diphenyl thiourea	5 ml. of 1% w/v soln. in 95% ethanol	Pt	P	P	P	P	P	P
		Pd	C	C	C	P	C	P
		Rh	P	P	P	S	P	S
		Ir	S	S	S	N	S	N
		Au	C	C	P	S	P	S
		Ni	N	N	N	N	N	N
		Cu	N	N	N	N	N	N
		Fe	N	N	N	N	N	N

[a] Nearly complete.
[b] Slight precipitate containing possibly a little iron.
[c] Not boiled.

of water when necessary. If a precipitate formed, the mixture was filtered and the filtrate tested for the metal.

The organic reagents are listed in Table I, with results for the metals investigated. The solid compounds were purified by crystallization from ethanol.

The spot tests used for platinum, palladium, iridium, and rhodium were discussed by Thompson, Beamish, and Scott (*13*). Stannous chloride was used for platinum, palladium, rhodium and gold, sulfuric and fuming nitric acids for iridium, dimethyl glyoxime for nickel, and ferrocyanide for iron and copper.

In many cases where complete precipitation was obtained gravimetric determinations for the metals were carried out. The precipitate and filter were ignited, reduced by burning in hydrogen if necessary, cooled in nitrogen if reduced, or in air, and weighed as the metal.

Some of the results for these determinations are listed in Table II. A number of the results were slightly high (typified by Nos. 1 to 3).

Phenyl thiourea, thiophenol, and thiobarbituric acid are suitable for the determination of palladium. Mann and Purdie (*11*) stated that thiophenol precipitated a $Pd(SPh)_2$ which decomposes to palladium quantitatively when heated, but they did not outline a method for its determination or record results. The results obtained for the determination of gold by thiophenol tend to be slightly high.

The platinum solution for determinations 1 to 3 was standardized by the thiophenol method, by the sodium formate method (*3*), and by precipitation with zinc (*2*). The palladium solutions were standardized by the potassium iodide method (*1*) and the gold solutions by the hydroquinone method (*3*).

Table II. Determination of Metals

Detn. No.	Reagent	Acid	Normality of Acid	Metal Determined	Metal Taken, Mg.	Metal Recovered, Mg.
1	*s*-Di-*o*-tolyl thiourea	HCl	0.60	Platinum	19.18	19.49
2	*s*-Di-*o*-tolyl thiourea	HCl	0.60	Platinum	19.18	19.51
3	*s*-Di-*o*-tolyl thiourea	HCl	0.60	Platinum	19.18	19.54
4	Phenyl thiourea	Aqua regia	0.012	Palladium	10.00	10.00
5	Phenyl thiourea	HCl	0.012	Palladium	10.00	10.00
6	Phenyl thiourea	HNO_3	0.012	Palladium	10.00	10.02
7	Phenyl thiohydantoic acid	HNO_3	0.60	Palladium	10.00	10.03
8	Thiophenol	HCl	0.012	Palladium	9.91	9.97
9	Thiophenol	HCl	0.012	Palladium	24.98	24.96
10	Thiophenol	HCl	0.012	Palladium	24.98	25.00
11	Thiophenol	HCl	0.012	Palladium	24.98	24.98
12	Thiophenol	HCl	0.012	Palladium	24.98	24.96
13	Thiophenol	HCl	0.012	Palladium	24.98	25.00
14	Thiophenol	HCl	0.012	Palladium	24.98	24.98
15	Thiobarbituric acid	HCl	0.012	Palladium	9.82	9.86
16	Thiobarbituric acid	HCl	0.06	Palladium	9.82	9.82
17	Thiobarbituric acid	HCl	0.06	Palladium	9.82	9.86
18	Thiobarbituric acid	HCl	0.30	Palladium	9.82	9.83
19	Thiobarbituric acid	HCl	0.30	Palladium	9.82	9.84
20	Thiophenol	Aqua regia	0.012	Gold	10.56	10.68
21	Thiophenol	HNO_3	0.012	Gold	10.56	10.53
22	Thiophenol	HNO_3	0.60	Gold	10.56	10.54
23	Thiophenol	HCl	0.012	Gold	10.56	10.66
24	Thiophenol	HCl	0.012	Gold	25.26	25.34
25	Thiophenol	HCl	0.012	Gold	25.26	25.32
26	Thiophenol	HCl	0.012	Gold	25.26	25.29
27	Thiophenol	HCl	0.012	Gold	25.26	25.37
28	Thiophenol	HCl	0.012	Gold	25.26	25.34
29	Thiophenol	HCl	0.012	Blank	0.00	−0.01
30	Thiophenol	HCl	0.012	Platinum	9.97	9.97
31	Thiophenol	HCl	0.012	Platinum	9.97	9.96
32	Thiophenol	HCl	0.012	Platinum	9.97	9.97
33	Thiophenol	HCl	0.012	Platinum	9.97	9.98
34	Thiophenol	HCl	0.012	Platinum	25.13	25.12
35	Thiophenol	HCl	0.012	Platinum	25.13	25.20
36	Thiobarbituric acid	HCl	0.03	Rhodium	25.08	25.09
37	Thiobarbituric acid	HCl	0.03	Rhodium	25.08	25.12
38	Thiobarbituric acid	HCl	0.03	Rhodium	25.08	25.08
39	Thiobarbituric acid	HCl	0.03	Rhodium	25.08	25.08
40	Thiobarbituric acid	HCl	0.03	Rhodium	25.08	25.07
41	Thiobarbituric acid	HCl	0.03	Rhodium	25.08	25.10
42	Thiobarbituric acid	HCl	0.03	Rhodium	25.08	25.09
43	Thiobarbituric acid	HCl	0.012	Rhodium	25.08	25.03
44	Thiobarbituric acid	H_2SO_4	0.036	Rhodium	25.08	25.16
45	Thiobarbituric acid	HCl	0.30	Rhodium	25.08	25.13

DETERMINATION OF PLATINUM

As a result of this investigation a new method was developed for the gravimetric determination of platinum by precipitation with thiophenol.

The platinum solution was prepared from pure platinum sponge which was dissolved in aqua regia, evaporated to dryness over a steam bath four times in the presence of concentrated hydrochloric acid, and finally dissolved in very dilute hydrochloric acid, filtered through a 7-cm. Whatman No. 44 paper, and washed. The filter paper was ignited, and the residue dissolved in aqua regia, evaporated to dryness four times with hydrochloric acid, dissolved in water, and filtered. The filtrates were diluted to the required volume and acidified to 1 ml. of concentrated hydrochloric acid per liter.

An aliquot of the solution containing approximately 10 to 25 mg. of platinum was diluted to 200 ml. and acidified with 4 drops of concentrated hydrochloric acid. One milliliter of a fresh 10% by volume solution of thiophenol in 95% ethanol was added to the cold solution, which was then boiled for 2 hours, filtered through a Whatman No. 44, 7-cm. filter paper, and washed with water. The paper and residue were permitted to drain and while still moist were transferred to a Coors 000 porcelain crucible and heated, commencing with a small flame. When smoke was no longer produced at that burner setting, the flame was gradually increased and the slow ignition was continued until the paper was thoroughly charred. The residue was then ignited at red heat for 20 minutes, cooled, and weighed. High results were obtained if the residue were ignited immediately at high temperature.

In order to facilitate the slow ignition of the residues a draft-free combustion chamber was made from Transite with holes along the bottom of each side to permit entry of air. It was fitted with a thin glass cover supported about 1 cm. above the top of the cupboard and a window for easy observation of the combustion. Upon evaporation of the filtrate platinum proved to be absent. Complete precipitation was obtained with an acid strength as great as 0.05 *N*, but a greater acid strength resulted in incomplete precipitation. Results appear in Table II (30 to 35).

The reagent tends to oxidize on exposure to air and incomplete precipitation is obtained with the partially oxidized reagent. The precipitate was a characteristic yellow-green color. When the oxidized reagent was used, or the reagent was added to a hot solution, the precipitate became orange-colored on boiling. The reagent may be kept in a small vial placed inside a large Nesbitt tower. The air is swept from the tower by means of a stream of nitrogen, and the tap on the tower is closed to keep the reagent in the nitrogen atmosphere. The reagent should be freshly prepared every 3 or 4 days. Five drops of thiophenol may be used instead of 1 ml. of the 10% thiophenol reagent.

DETERMINATION OF RHODIUM

A method was developed for the gravimetric determination of rhodium by precipitation with thiobarbituric acid.

The rhodium solution was prepared by dissolving a sodium salt of rhodium chloride in water containing 0.5 ml. of hydrochloric acid, filtering, washing thoroughly, and diluting to 2 liters. (The "sodium rhodium chloride" was donated by the International Nickel Company of Canada.) The solution was standardized by the hydrogen sulfide method (*9*). An aliquot of solution containing approximately 25 mg. of rhodium was diluted to 200 ml. and acidified with 0.5 to 5 ml. of hydrochloric acid, 10 ml. of 1.4% w/v thiobarbituric acid in 95% ethanol were added, and the solution was heated to boiling. The pink solution became a dark red-brown in color. After boiling 2 to 3 minutes a very fine red-brown precipitate appeared. The solution was boiled for 2 hours and the precipitate settled out as chocolate-brown crystals. The mixture was filtered, washed, ignited at red heat for 20 minutes, reduced in hydrogen, cooled in nitrogen, and weighed. Results are included in Table II (36 to 45).

Spot tests on the evaporated filtrates proved the absence of rhodium. This method presents greater ease of operation than the hydrogen sulfide method. The results obtained by both methods agree very closely. Thiobarbituric acid is the first organic precipitant proposed for the gravimetric determination of rhodium.

An attempt was made to determine the composition of the rhodium–thiobarbituric acid complex.

A rhodium precipitation was carried out in ethanol with thiobarbituric acid reagent. The precipitate was filtered, washed with ethanol, dried under reduced pressure, and weighed. The residue was transferred to a small beaker, the organic matter destroyed with sulfuric and nitric acids, and the rhodium determined by the hydrogen sulfide method. The percentage of rhodium in the complex averaged from three determinations was 26.7%.

If two molecular weights of thiobarbituric acid were combined with one atomic weight of rhodium, the latter replacing one hydrogen of each thiobarbituric acid molecule, the amount of rhodium present would be 26.5%. If one rhodium were associated with three thiobarbituric acid molecules the amount of rhodium present would be 19.3%. The valence of 2 for rhodium corresponds to the usual valence for other metals with organic compounds of this type but is an uncommon valence for rhodium.

SUMMARY

With the organic monosulfide compounds used in this investigation, palladium and gold seem to be most readily precipitated completely under the conditions described. However, platinum forms precipitates completely in many cases. It is more difficult to precipitate rhodium and iridium. Copper, nickel, and iron do not form precipitates readily with these reagents.

Conditions are described for the gravimetric determination of rhodium by thiobarbituric acid and of platinum by thiophenol.

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Hexamethylenetetramine in Separations of Titanium and Columbium

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A procedure for gravimetric determination of titanium in ferrotitanium alloys is reported, in which hexamethylenetetramine eliminates the use of hydrogen sulfide for removal of iron. Sodium hydroxide and hexamethylenetetramine are used in a procedure for determining columbium, titanium, or both in high chromium-nickel steels containing tungsten, vanadium, and molybdenum. The procedures are believed to be applicable to determination of zirconium.

THE use of hexamethylenetetramine has been developed in the following procedures to separate divalent iron from titanium and columbium.

Lehrman (*4*, *5*) used hexamethylenetetramine qualitatively to separate divalent ions from numerous trivalent and tetravalent ions. Specific quantitative procedures using the reagent have been developed (*1*, *2*, *3*, *6*, *7*) to separate trivalent iron, aluminum, chromium, and titanium from zinc, nickel, cobalt, manganese, calcium, and magnesium.

These references indicated a method of separating iron from titanium and columbium contained in ferro-alloys or stainless steels, based on reduction of iron to the divalent state. The iron of those alloys and steels that may require an oxidizing acid for solution can be readily reduced by zinc after removal of the oxidant. Two procedures have been developed: one for the gravimetric determination of titanium in ferrotitanium alloys, the other for the determination of titanium and columbium in high chromium-nickel steels.

REAGENTS

All reagents used were the usual C.P. grade. Hexamethylenetetramine was purchased from the Eastman Kodak Company. Zinc spirals were obtained from the G. Frederick Smith Company, Columbus, Ohio.

GRAVIMETRIC DETERMINATION OF TITANIUM IN FERROTITANIUM ALLOYS

In a reduced solution hexamethylenetetramine will precipitate titanium and leave the iron in solution, presenting many advantages over the hydrogen sulfide method for the removal of iron:

1. It eliminates the use of obnoxious hydrogen sulfide and permits the operations to be performed on the working bench without any unusual ventilation.
2. The desired element, titanium, is precipitated, thus removing any possible loss by hydrolysis.
3. After a hydrogen sulfide separation the filtrate must be boiled to remove this reagent. It has been the experience of the author that the sulfur residue has always contained a trace of titanium that requires an additional operation to recover.
4. Titanium precipitated with hexamethylenetetramine will filter much faster than the bulky iron sulfide precipitate.
5. No precipitates or residue requires working in platinum. This eliminates the necessity of removing traces of platinum.

Any zirconium will be found with the titanium, and appropriate means for its determination may be taken.

Many common separations have been used to complete the desired analysis, such as cupferron and sodium hydroxide, and are mentioned only by name. Complete details can be found in any standard reference.

Procedure A. Place 0.5000 gram of 80-mesh alloy in a 400-ml. beaker, add 100 ml. of 5% sulfuric acid, and heat gently until the reaction has ceased. Add nitric acid dropwise until the sample is oxidized. Make a sodium hydroxide separation to remove vanadium. Transfer the washed precipitate and paper to the beaker in which it was made. Destroy the organic matter with 15 ml. of sulfuric acid, 25 ml. of nitric acid, 10 ml. of perchloric acid (70 to 72%), and 5 to 8 grams of sodium sulfate by covering and evaporating to fumes of sulfuric acid. Make sure that all the perchloric acid has been removed. Cool and dilute the sample to 150 ml. with water. Heat the sample until all the salts are in solution, introduce an amalgamated zinc spiral, and reduce the solution. Since all the iron will reduce before any titanium, the deep violet color of reduced titanium will indicate the complete reduction of iron to the divalent state.

Remove the spiral and add 3 to 4 drops of 10% aerosol (wetting agent obtained from any chemical supply house). By lowering the surface tension of the solution the air oxidation of divalent iron is eliminated. Add ammonium hydroxide dropwise until the black precipitate that forms just redissolves. Add a 10% solution of hexamethylenetetramine until the sample is alkaline to methyl red, introduce a little ashless paper pulp, and

Table I. Determination of Titanium

B. of S. Sample	Ti Contained %	Ti Found %
	Procedure A	
116	25.48	25.43 25.50
116A	25.08	25.09 25.05 25.08 25.11
	Procedure B	
116	25.48	25.53
116A	25.08	25.10 25.13 25.10[a] 25.14[b]

[a] Contained 0.25 gram of V.
[b] Contained 0.5 gram of V.

oil for 1 minute. Filter on a medium texture paper using a moderate suction, and wash the precipitate 5 to 6 times with a hot 0.5% hexamethylenetetramine solution.

Return the precipitate and paper to the beaker and destroy the organic matter as described above. After the perchloric acid has been removed, cool, dilute the sample to 100 ml. with water, heat to boiling, and filter. Wash the residue with hot water, and discard. (It will be silicic acid with possibly some aluminum oxide.) Heat the filtrate and reduce it with a zinc spiral. Repeat the ammonium hydroxide and hexamethylenetetramine separation.

If the sample contains copper, destroy the hexamethylenetetramine precipitate and paper as above. Dilute the sample to 100 ml. with water and make an ammonium hydroxide separation with a 5% excess. Boil, filter, and wash the precipitate with a hot 5% ammonium hydroxide solution.

Transfer the precipitate and paper to the same beaker and digest it with 30 ml. of sulfuric acid. Dilute to 100 ml. with hot water and stir until the precipitate is completely in solution. Cool the sample to 10° C. and make a cupferron separation. Place the cupferron precipitate in a tared platinum dish of 75-ml. capacity, dry, and ignite with the usual precautions to a constant weight. In umpire work correct the titanium dioxide for traces of silica and iron. Convert the corrected weight of titanium dioxide to titanium (Table I, A).

Inasmuch as vanadium can be reduced to a valence of 2 with zinc, it was decided to investigate the possibility of eliminating the sodium hydroxide separation for its removal. This was accomplished with a considerable saving of time. Iron and titanium must be reduced to a valence of 2 and 3, respectively, before vanadium will reduce to a valence of 2. If the operator finds it difficult to determine the complete reduction of the sample, Procedure A is suggested.

Procedure B. Place 0.5000 gram of the alloy in a 400-ml. beaker with 100 ml. of 10% sulfuric acid and heat until the reaction has ceased. Introduce a zinc spiral to ensure complete reduction (15 to 30 minutes). Add 3 to 4 drops of aerosol and proceed with the double hexamethylenetetramine and ammonium hydroxide separations as given in Procedure A. Precipitate the titanium with cupferron, dry, and ignite the precipitate to a constant weight (Table II, B). Vanadium was added to two Bureau of Standards No. 116A samples as indicated.

DETERMINATION OF TITANIUM AND COLUMBIUM IN HIGH CHROMIUM-NICKEL STEELS

In the usual procedure for the determination of columbium in heat-resisting steels this element is hydrolyzed in a dilute acid solution. If the steel also contains an appreciable amount of titanium this method is not very satisfactory. Titanium can be hydrolyzed, but it is a troublesome and time-consuming analysis. When, in addition, tungsten, vanadium, and molybdenum are alloyed in the steel, the determination of columbium and titanium is further complicated.

In the author's method tungsten, vanadium, and molybdenum are removed by a sodium hydroxide separation. While it is true that columbium alone is only partially precipitated by sodium hydroxide, the results indicate that in the presence of titanium and iron, columbium can be successfully separated from tungsten and vanadium. It appears that columbium like titanium requires the presence of iron to ensure complete precipitation in a sodium hydroxide separation. It is very important that the sodium hydroxide precipitate be washed with a 2% sodium hydroxide solution. Washing the precipitate with hot water causes a loss of columbium. Molybdenum is one of the elements with a valence higher than 2 that is not precipitated by hexamethylenetetramine. If the steel contains no tungsten or vanadium but only molybdenum, no sodium hydroxide separation is necessary for its removal. Two hexamethylenetetramine separations removed the molybdenum completely from an alloy of molybdenum and titanium containing over 50% molybdenum.

If the sample contains tantalum it will be found with the columbium and titanium, and appropriate means for its determination must be taken. The developed procedure will give columbium, titanium, or both if they are present in the sample.

Procedure. Place 5.000 grams of drillings in 400-ml. beaker, add 100 ml. of 1 to 2 hydrochloric acid, and heat until the reaction has ceased. Cool the sample to 10° C. and precipitate the columbium and titanium by the usual cupferron method for titanium in steels. Be sure to precipitate sufficient iron so that columbium and titanium will not be lost in the subsequent sodium hydroxide separation (25 ml. of a 6% solution should be more than sufficient). Return the precipitate and paper to the beaker. Destroy the organic matter with 10 ml. of sulfuric acid, 25 ml. of nitric acid, 10 ml. of perchloric acid, and 3 grams of sodium sulfate, by covering and evaporating to fumes of sulfuric acid. Cool, dilute the solution with water, and make a sodium hydroxide separation to remove the tungsten and vanadium. (If no tungsten or vanadium is present omit this separation.) Wash the precipitate moderately with a hot 2% sodium hydroxide solution.

Transfer the precipitate and paper to the same beaker and destroy the organic matter as in the cupferron precipitate, making sure that all the perchloric acid is removed. Cool, dilute the sample to 150 ml. with water, add 5 ml. of hydrochloric acid, and heat. Reduce the iron with a zinc spiral (indicated by the disappearance of the yellow color due to ferric chloride). Add 3 to 4 drops of aerosol and ammonium hydroxide dropwise until the solution is nearly neutral to methyl red. The appearance of a white precipitate will be due to titanium or columbium hydroxide and is of no consequence to the removal of the iron. Add 10% hexamethylenetetramine solution until the sample is alkaline to methyl red. Introduce some ashless paper pulp and boil the sample for 1 minute. Filter and wash the precipitate 5 times with a hot 0.5% hexamethylenetetramine solution. Destroy the organic matter in the precipitate and paper as given for the cupferron precipitate. Repeat the ammonium hydroxide and hexamethylenetetramine separation.

Table II. Determination of Titanium and Columbium

B. of S. Sample	% Contained	% Found
121	0.394 Ti	0.403 Ti[a] 0.400 Ti[a]
123A	0.75 Cb	0.78 Cb[b] 0.78 Cb[b]
		0.356 Ti 0.77 Cb[b]
121A, 2.5 grams 123A, 2.5 grams	0.361 Ti 0.75 Cb	0.352 Ti 0.78 Cb[b]
		0.354 Ti 0.77 Cb[b]

[a] Weighed as TiO_2.
[b] Not corrected for Ta. Sample contains 0.02% Ta.

Transfer the precipitate and paper to the same beaker and digest it with 30 ml. of 1 to 1 sulfuric acid. Dilute the sample to 100 ml. with hot water and stir well. Cool the solution to 10° C. and make a cupferron separation. Dry and ignite the precipitate in a tared platinum dish of 75-ml. capacity. Remove silica in the usual manner with sulfuric and hydrofluoric acids.

The residue is composed of the mixed oxides of columbium and titanium. Fuse the residue with sodium bisulfate. Dissolve and determine the titanium colorimetrically with hydrogen peroxide in a 5% sulfuric acid solution. Subtract the weight of titanium dioxide from the total weight of the oxides. Convert the difference, columbium pentoxide, to columbium (Table II).

DISCUSSION

When checked by spectrographic methods the precipitates showed no mercury or zinc, indicating that there is no contamination from the amalgamated zinc spirals.

In the gravimetric determination of titanium in ferrotitanium alloys, a procedure has been developed using hexamethylenetetramine that eliminates the use of obnoxious hydrogen sulfide for the removal of the iron.

In the determination of columbium and titanium, sodium hydroxide and hexamethylenetetramine are used to determine columbium, titanium, or both in high chromium-nickel steels containing tungsten, vanadium, and molybdenum.

Preliminary investigation on synthetic mixtures of iron and zirconium indicates that the procedures are applicable to the determination of zirconium.

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Determination of Metallic Copper in Cuprous Oxide-Cupric Oxide Mixtures

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For the determination of metallic copper in cuprous oxide–cupric oxide mixtures, the cuprous oxide is dissolved in a dilute alcoholic hydrochloric acid solution and the metallic copper is determined in the insoluble metallic copper–cupric oxide residue by direct solution in acid ferric chloride followed by titration with ceric sulfate. Reaction between cupric ions and metallic copper is prevented by addition of stannous chloride which reduces the cupric ion to cuprous ion. Difficulties in the analysis of commercial copper and cuprous oxide pigments, due to physical characteristics of the pigments, are presented. The effects of exposure time, temperature, atmosphere, and solvents are listed.

A large number of analyses were made by this method on synthetic mixtures and commercial copper and cuprous oxides with excellent results. The method is rapid and accurate and requires only the apparatus found in the average laboratory. The personal factor involved in most attempts to develop a method of analysis is eliminated and only a few and relatively simple precautions are required in the performance of the analysis.

COMMERCIAL cuprous oxide and copper pigments for use in antifouling paints or insecticides are prepared by several methods. The electrolytic method for the preparation of cuprous oxide is based on the anodic oxidation of copper in an alkaline solution. By control of the current density and the temperature of the alkaline salt solution, the particle size of the cuprous oxide can be controlled to produce a cuprous oxide of high purity. Pyrometallurgical cuprous oxide is prepared by the reduction of mixed copper oxides obtained (*a*) as a by-product of wire drawing or copper annealing operations, (*b*) from the copper recovery of ore tailings, (*c*) by leaching scrap copper and rich ore concentrates, and (*d*) from cement copper powder. The mixed copper oxides are reduced by heating in a reducing atmosphere to change the cupric oxide to cuprous oxide and grinding to a controlled fineness. The cuprous oxides are then coated with a material such as stearic acid, protein, pine oil, or mineral oil to prevent oxidation on storage. A new type of copper pigment for use as a substitute for cuprous oxide in antifouling paints is prepared from cement copper, which is obtained as a sludge from acid copper mine water which has been passed over iron. The copper is displaced from solution by the iron, forming a spongy precipitate of copper particles. Each particle of copper is coated with a layer of cuprous oxide.

The particle sizes of the constituents of the copper and cuprous oxide pigments are in general nonuniform and vary from 0.2 to 30 microns with a mean average diameter ranging from 1 to 5 microns. The pigment obtained from these manufacturing operations contains various percentages of cupric oxide and metallic copper, depending on the processing received and the raw material used. Besides variations in composition, the various manufacturing processes result in different particle shapes and surface structure. In some types the cuprous oxide particles are covered with finer particles of cupric oxide. By electron microscopic photographs, the particles of cupric oxide formed in the pigment on aging or as a result of incomplete reduction in manufacture were found to be approximately one tenth the size of the cuprous oxide particles in the pyrochemical type cuprous oxide. In the copper pigments, the metallic copper contains cuprous oxide on the surface of the copper. The electrolytic pigments have particles of definite structure, whereas the pyrometallurgical pigments have irregular shaped particles. The data presented in Table I indicate the wide variation in physical properties of these pigments.

LITERATURE REVIEW

In a previous paper (*1*) the authors reviewed the difficulties involved and the results of numerous attempts to develop a quantitative determination of the components of the finely divided mixtures of cuprous oxide, cupric oxide, and metallic copper. A method was developed that gave reproducible results on a number of types of electrolytic and pyrometallurgical cuprous oxide pigments. The procedure is based on the use of a dilute aqueous hydrochloric acid solution, using hydrazine sulfate to reduce any dissolved cupric ions. Although an improvement over previous methods, further experiments have shown the method to lack sufficient accuracy. Since the publication of this paper, a number of manufacturers have undertaken the manufacture of cuprous oxide and copper pigments. Samples received from several manufacturers were analyzed, using the hydro-

Table I. Physical Properties of Cuprous Oxide and Copper Pigments

Grade	Electrolytic Cu_2O	Copper Pigment	Pyrometallurgical Cu_2O Manufacturer 1	Pyrometallurgical Cu_2O Manufacturer 2
Apparent density, lb./cu. ft.	51.2	97.8	128.8	105–132
Oil absorption, lb. of oil/100 lb. of pigment	11.7	15.2	8.1	6.5–7.1
Specific gravity	5.59		5.72	5.84–5.94
Color (Munsell)	R 4/6	R 3/6	R 4/6	R 3/4–R 4/6
Crystal structure	Star shaped	Porous	Irregular	Irregular
Average particle diameter, microns	2.1	2.5	2.1	3.0–5.0
% particles less than 1 μ	11	33	60	15–22
% particles 1–3 μ	56	46	32	19–40
% particles 3–5 μ	25	17	6	10–23
% particles over 5 μ	8	4	2	21–49

chloric acid–hydrazine sulfate method (*1*) for metallic copper content.

Negative figures are obtained for cupric oxide and two of the samples show over 100% of cuprous oxide plus metallic copper. It is believed that this is indicative of: high total reducing power, presence of other metals or reducing agents having a reducing action on ceric sulfate, or low metallic copper.

Previous results (*1*) on the determination of total reducing power using ceric sulfate showed the accuracy of this method to be satisfactory. Examination of the pigments showed that no other metals or organic matter are present in sufficient quantity to affect the results materially. Therefore, the indication is that the metallic copper is low.

DEVELOPMENT OF METHOD

One of the chief difficulties involved in developing an analytical procedure for the determination of copper in the presence of cuprous oxide and cupric oxide is in the preparation of a standard reference sample, the composition of which is definitely known. A standard prepared by mixture of a pure electrolytic cuprous oxide, copper powder, and cupric oxide is not representative of the commercial pigments. The cupric oxide in the synthetic mixtures has larger particle size and different surface structure, and is less reactive than the cupric oxide in the pigment which is often formed directly on the copper or cuprous oxide particles. The commercially prepared pigment is an intimately mixed combination of the copper cuprous oxide, and cupric oxide prepared simultaneously in situ during the manufacturing process, whereas the synthetic mixtures are composed of three definite, separate particles of copper, cuprous oxide, and cupric oxide. Moreover, the physical properties of various grades of commercial pigments are sufficiently different to alter their reactivities toward various chemical reagents. Therefore, a procedure for the determination of copper must be accurate not only for synthetic mixtures but also for all types of commercial pigments before being acceptable. Negative figures for cupric oxide and total concentrations of ingredients adding to more than 100% will aid in determining the accuracy of a procedure. Reproducibility of results cannot be used as a criterion of accuracy, but only as a determination of the personal factor involved in the method.

As previously noted, the difficulty in selectively dissolving cuprous oxide and separating it from metallic copper in the presence of cupric oxide is based on the sensitivity of the finely divided components to chemical solutions and the effect of dissolved cupric ion on metallic copper. In the previous methods, the reagents used for dissolving cuprous oxide also attacked the copper, attacked the cupric oxide without reducing the resultant cupric ions rapidly enough to prevent attack on copper, or formed reaction produets that attacked the copper.

An indication of the effect of the reagent on the copper can be obtained by noting the effect of the time of contact between the reagents and the sample on the percentage of copper determined. In all previous methods, the time factor is of considerable importance, indicating that the metallic copper determinations are low. Maximum indication of accuracy is inferred from a procedure having a negligible time factor.

A reagent to be suitable must have no effect on metallic copper and slight attack on cupric oxide and must completely dissolve the cuprous oxide. A reagent that has no effect on cupric oxide is unsuitable for the determination of metallic copper in certain pyrometallurgical grade cuprous oxides, since a large portion of the cuprous oxide particles are covered with cupric oxide, thus preventing the required dissolving action. Too great an attack on the cupric oxide results in an excess concentration of cupric ions which may attack copper before reduction. Dilute hydrochloric acid (*1*) results in rapid and complete solution of the cuprous oxide and a slight attack on cupric oxide. Ammonium hydroxide, ammonium chloride, silver sulfate, and iodine were found to be unsuitable (*1*).

Table II. Conductivity of Aqueous and Organic Solutions

Solvent	Composition of Solutions per 100 Ml. of Solvent: Stannous chloride	Hydrochloric acid (sp. gr. = 1.19)	Conductivity 25° C.	Conductivity −10° C.
	Grams	*Ml.*	*Millimhos/cm.*	
Water	...	4	180	...
Water	4	4	190	...
Alcohol (95%)	...	4	7.4	4.8
Alcohol (95%)	4	4	6.6	3.6
Acetone	...	4	3.8	1.4
Acetone	4	4	8.0	6.5

Experiments were conducted to eliminate the attack of dilute hydrochloric acid on metallic copper and minimize the attack on cupric oxide. The strength of an acid is dependent on the degree of ionization, which is in turn dependent on the dissociation of the acid. Acids require water or similar polar mediums to enable ionization. Maximum ionization is obtained at infinite dilution. Although use of concentrated acid will decrease the total per cent ionization, its attack on the copper will increase. Therefore, the acid concentration is kept low but the water is replaced with an organic medium, ethyl alcohol, which reduces the ionization and conductivity of the solution. Alcoholic solutions of hydrochloric acid were found to give higher metallic copper figures, showing a decreased solution of copper. However, the results obtained for metallic copper decreased with increasing cupric ion content of the solution, indicating the need for a reducing agent to reduce any cupric oxide dissolved.

The selection of a reducing agent is restricted to a reagent soluble and active in dilute alcoholic hydrochloric acid solutions. After examination of a number of reducing agents, stannous chloride was found to meet the requirements. It is soluble in the alcoholic solution and quickly reduces cupric ion to cuprous ion. Further experiments on a number of commercial cuprous oxide samples containing very finely divided copper and cupric oxide showed that more accurate results and a decreased effect of time factor could be obtained with this solution by keeping the temperature low, particularly in cement copper pigments. The combined effect of lower temperature and inert atmosphere can be obtained by adding dry ice (solid carbon dioxide) to the reagent. Table II lists the conductivities obtained at various compositions of solutions and temperatures and illustrates clearly the reduced activity of the alcoholic solution.

REAGENTS

Denatured Alcohol, Formula 12A. To 1 liter of 95% ethyl alcohol, add 5 ml. of benzene. (Pure grain alcohol can be used in place of the denatured alcohol.)

Extraction Solution. Add 40 ml. of concentrated c.p. hydrochloric acid (sp. gr. 1.19) to 1 liter of the denatured alcohol. Mix thoroughly. Add 40 grams of c.p. fresh stannous chloride ($SnCl_2.2H_2O$) and stir until completely dissolved.

Ferric Chloride Solution. Add 75 grams of c.p. ferric chloride hexahydrate and 150 ml. of c.p. hydrochloric acid (sp. gr. 1.19) to 400 ml. of distilled water. Stir to complete solution, add 5 ml. of 30% hydrogen peroxide solution, and boil to remove excess.

Ceric Sulfate Solution. Mix 50 grams of ammonium hexanitrato cerate and 52 ml. of 1 to 1 sulfuric acid and dilute slowly to 1 liter with distilled water, stirring constantly. Allow to stand several days and filter off any suspended insoluble matter. Standardize by dissolving pure, analyzed copper foil free from oxide coatings in ferric chloride solution and titrating with the ceric sulfate solution, using *o*-phenanthroline indicator.

Indicator. *o*-Phenanthroline ferrous complex (ferroin), manufactured by G. Frederick Smith Co., Columbus, Ohio.

PROCEDURE

Add about 20 ml. of 4-mm. diameter glass beads to a 250-ml. Phillips conical beaker with lip. Weigh accurately, using tared glazed paper, a sample of suitable size depending on the metallic copper content. Use 1-gram sample for samples containing up to 10% metallic copper, 0.15-gram sample for high copper samples (80% metallic copper), and proportional amounts for intermedi-

ate mixtures, so that a titration greater than 50 ml. of the 0.1 *N* ceric sulfate is not required. Add 25 ml. of denatured alcohol to the beaker and swirl vigorously for 1 minute to disperse the pigment and break up any lumps. Add slowly 100 ml. of the extraction solution while swirling the flask. After the addition of the extraction solution, swirl the flask vigorously for 5 minutes, adding lumps of dry ice continuously during this time to lower the temperature of the solution to approximately 0° C. Break up any remaining lumps with a glass rod or policeman.

Filter off the metallic copper–cupric oxide residue on a filter pad as described below. Continue the addition of dry ice to the solution in the extraction flask to keep the temperature low. Wash the flask and residue with 150 to 200 ml. of denatured alcohol, using suction. Transfer the filter pad containing the residue to the original extraction flask and dissolve in 25 ml. of the ferric chloride–hydrochloric acid solution, maintaining an atmosphere of carbon dioxide above the sample by addition of dry ice. Heat on a steam bath to dissolve the copper. Add 50 ml. of distilled water and 3 drops of *o*-phenanthroline indicator. Titrate with the standard ceric sulfate until the color changes from orange to pale green. Calculate the metallic copper content as follows:

$$\frac{\text{Grams Cu per ml. ceric sulfate solution} \times \text{ml. ceric sulfate}}{\text{weight of sample}} \times 100 = \% \text{ metallic copper}$$

NOTES ON PROCEDURE

During the 5-minute swirling period, approximately 25 to 30 grams of dry ice are added in about 5-gram portions. The initial lumps of dry ice are volatilized rapidly, owing to the temperature of the solution. About 15 grams of dry ice are added in the first 2 minutes with a subsequent drop in temperature to 0° C. The remaining 10 grams volatilize more slowly and gradually lower the temperature to the vicinity of −10° C. These directions are not critical but merely serve as a guide line. During the filtration period, additons of dry ice to the flask should be continued to keep the solution cold until all the extraction solution has been filtered.

Table III. Determination of Metallic Copper in Synthetic Mixture

Weight Taken					
Metallic Cu[a]	Cu_2O[b]	CuO[c]	Cu Taken	Cu Found	Error
Gram	*Gram*	*Gram*	%	%	%
0.1250	...	..	...	98.8	....
0.0984	...	..	...	98.6	....
0.1402	...	..	...	98.5	....
....	0.100	..	...	0.48	....
....	0.100	..	...	0.50	....
....	0.100	..	...	0.50	....
....	...	1.00	...	0.0	....
....	...	1.00	...	0.0	....
0.0195	0.980	..	2.42	2.38	−0.04
0.0143	0.985	..	1.90	1.87	−0.03
0.0257	0.975	..	3.03	2.57	−0.06
0.0789	0.910	..	8.34	8.22	−0.08
0.1405	0.860	..	14.31	14.19	−0.12
0.1410	0.400	..	26.1	25.9	−0.20
0.1329	0.130	..	50.3	50.1	−0.20
0.1307	0.050	..	71.6	71.5	−0.1
0.0205	0.810	0.20	2.01	1.98	−0.03
0.0413	0.820	0.20	4.23	4.25	+0.02
0.0745	0.750	0.20	7.22	7.19	−0.03
0.1426	0.610	0.20	15.1	14.9	−0.20
0.0317	0.510	0.50	3.25	3.27	+0.02
0.0787	0.500	0.50	7.44	7.31	−0.13
0.1384	0.420	0.50	13.1	12.9	−0.20
0.1211	0.130	0.10	34.3	34.2	−0.10
0.1148	0.050	0.05	52.9	51.7	−0.40
0.1445	...	0.05	73.4	73.2	−0.20
0.1327	...	0.17	43.3	43.4	+0.10
0.1126	...	0.46	19.6	19.5	−0.10
0.1098	...	0.75	12.6	12.4	−0.20
0.1275	...	0.88	12.5	12.4	−0.10

[a] 325-mesh copper powder extracted with extraction solution used in procedure. Total reducing power of extracted copper powder is 222.3, equivalent to 98.8% Cu. This figure was used for metallic copper for calculation of % Cu taken.

[b] A commercial grade material containing 0.5% metallic Cu. Calculated copper was corrected for this amount.

[c] A 325-mesh reagent grade material with a 0.0% total reducing power.

Table IV. Analyses of Commercial Copper and Cuprous Oxide Pigments

Lot No.	29	1110	T-675	926	656	237	252H	3	1
Type	Grade A Cu_2O	Grade B Cu_2O	Copper Pigment	Grade A Cu_2O	Grade B Cu_2O	Grade B Cu_2O	Grade A Cu_2O	Copper Pigment	Copper Pigment
Copper, %	0.69	9.33	77.3	0.55	5.67	2.97	4.39	80.3	75.7
	0.71	9.46	77.1	0.61	5.50	2.94	4.30	80.1	76.0
	0.68	9.51	77.0	0.58	5.61	2.96	4.33	80.5	75.8
	0.71	9.55	77.4	0.58	5.60	2.90	4.29	80.5	75.6
	0.70	9.43	77.3	0.60	5.55	2.95	4.18	80.3	75.9
	0.68	9.50	77.4	0.61	5.65	2.90	4.35	80.4	76.0
Av.	0.69	9.45	77.3	0.59	5.60	2.94	4.31	80.4	75.8
Av. deviation, %	±0.01	±0.05	±0.1	±0.03	±0.05	±0.04	±0.05	±0.1	±0.1
Total reducing power	99.4	101.1	188.9	96.0	101.3	92.0	101.4	190.0	186.6
Total copper (electrolytically)	87.8	88.1	91.6	87.4	88.4	80.6	86.8	88.6	90.5
Cu_2O, %	97.8	79.8	15.0	94.7	87.7	85.3	91.7	9.1	16.0
CuO, %	0.3	9.7	1.3	3.4	6.1	2.4	1.3	0.2	0.7

The electrolytic type of cuprous oxide pigment (252H) was the only one which gave some difficulty in dispersing and breaking up lumps. The difficulty is due to the fact that a different coating is used on this pigment to prevent air oxidation. After swirling 5 minutes with the extraction solution, a number of small, undispersed lumps of cuprous oxide were clearly visible. However, these can be readily dispersed and dissolved by crushing them with a glass rod or a policeman. The solution should not be filtered until there is no evidence of lumps present.

Because of the extremely fine particle size of the metallic copper in some samples, care must be exercised in the filtration. The following method of filtration has been found satisfactory:

Prepare a layer of coarse asbestos on a glass or hard-rubber perforated disk contained in a Shimer filtering tube. Next, place a layer of fine asbestos over the coarse and pack firmly using suction. Wash the filter pad thoroughly with denatured alcohol. The pad is now ready for the filtration. If any copper passes through the pad, as is evidenced by a reddish color in the filtrate, discard the sample and repeat the analysis using a thicker and more tightly compressed pad.

RESULTS OF ANALYSIS

The metallic copper content of a number of synthetic mixtures was determined by the above procedure (Table III). The synthetic mixtures were prepared by weighing various amounts of metallic copper, cuprous oxide, and cupric oxide in such proportions as to obtain from 0 to 100% of each ingredient, to determine the effect of each constituent on the result for metallic copper. In only one case was the error greater than 0.3%. Although these results are satisfactory, it must be borne in mind that the ingredients were prepared separately and are not the same intimate mixtures that are found in commercial samples. Moreover, the copper powder is not so fine as many of the metallic copper particles found in the commercial samples.

Table IV lists the results of metallic copper determinations on a number of commercial antifouling pigments. Lots 29, 1110, 926, 656, and 237 are pyrometallurgical-type cuprous oxide pigments. Lot 252H is an electrolytic cuprous oxide. Lots T-675, 1, and 3 are copper pigments from cement copper. The average deviations are indicative of the degree of accuracy obtainable with this procedure. The total reducing powers and the total coppers were determined by the methods outlined in (*1*), except that the ceric sulfate was standardized with C.P. analyzed copper foil. The cuprous oxide and cupric oxide were determined by calculation. The increased accuracy of the method is indicated by the results obtained for lot 3. All the results for metallic copper obtained by methods previously discussed gave a considerably lower figure. This resulted in a negative figure for cupric oxide—for example, the method of (*1*) gave a figure of 70% metallic copper for lot 3 which resulted in a negative cupric oxide value of −12.9%. The average deviations obtained for the samples containing less than 10% copper was ±0.05%. For high copper samples, an average deviation of ±0.1% was obtained.

EXPERIMENTAL WORK

Effect of Contact Time and Temperature. The time of shaking in the extraction solution was varied from 2 minutes to 1 hour, maintaining the low temperature by continual additions of dry ice. High results were obtained on lots 926 and 29 when shaken only 2 minutes. Check results were obtained with all the samples when shaken in the extraction solution for from 5 minutes to 1 hour, provided the temperature was kept low. The effect of the decreased temperature is to eliminate the solvent action of the solution on the finely divided copper and greatly decrease the solvent action on the cupric oxide.

Effect of Atmosphere and Temperature. The determination of copper in an aqueous medium requires the use of an inert atmosphere above the solution to prevent rapid oxidation due to the high oxygen sensitivity of an aqueous acid solution of copper. The experiments with the proposed method show that the copper in the organic solution is insensitive to oxygen and the presence of an inert atmosphere is unnecessary. The important factor in the use of the alcoholic extraction solution, as demonstrated in Table V, is the use of low temperatures. Excellent results are obtained at low temperatures regardless of the surrounding atmosphere, and despite the fact that the copper is kept in contact with the extraction solution for one hour before filtering. At room temperature, however, low results are obtained in most cases when the copper and extraction solution are in contact one hour regardless of the atmosphere.

Effect of Solvent. The choice of a suitable solvent is limited by several factors. The solvent must remove the surface coating to disperse the pigment. The stannous chloride and cuprous chloride formed from the reaction of cuprous oxide and hydrochloric acid must be completely soluble in the solvent. These conditions eliminate most of the solvents except acetone and alcohol. The use of acetone results in rapid deterioration of the extraction solution and yields low metallic copper figures on the samples of high metallic copper content. The mixture of hydrochloric acid, stannous chloride, and denatured alcohol remains clear and water-white indefinitely. Replacing the alcohol with acetone results in the formation of a light yellow reaction product in 24 hours and a dark brown reaction product in several weeks. The result for metallic copper becomes progressively lower as the acetone extraction solution ages. No deleterious aging effects are found in the alcohol extraction solution.

Table V. Effect of Temperature and Atmosphere on Metallic Copper Determinations

Lot No.	29	656	252H	3
−10° C. with dry ice additions to the solution	0.70	5.63	4.30	80.2
−10° C. by placing extraction flask in alcohol–dry ice bath (no inert atmosphere)	0.69	5.58	4.35	80.5
CO_2 gas (room temperature)	0.52	4.67	4.25	79.1
N_2 gas (room temperature)	0.55	4.40	2.98	78.7
Room temperature (no inert atmosphere)	0.40	3.77	3.07	78.5

Samples remained in contact with extracting solution for 1 hour.

Acid Concentration. The concentration of 4 ml. of concentrated hydrochloric acid per 100 ml. of solvent used in the extraction solution represents the minimum quantity that will completely dissolve all the cuprous oxide in the samples tested. Doubling the acid concentration does not materially affect the results, owing to the low conductivity of the solution.

Stannous Chloride Concentration. Less than 2 grams or more than 6 grams of stannous chloride per 100 ml. of denatured alcohol in the extraction solution results in low metallic copper figures in a number of instances.

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The views presented in this article are those of the writers and are not to be construed as the official views of the Navy Department.

Determination of Magnesium in Aluminum Alloys

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The authors submit an analytical method of making the initial separation of magnesium hydroxide in the presence of other alloying constituents held in solution as complexes formed under controlled pH conditions. Final determination of magnesium may be a simple spectrophotometric measurement of the light transmission or optical density of the magnesium hydroxyquinolate in hydrochloric acid solution, the volumetric bromate-thiosulfate titration of magnesium hydroxyquinolate, or a gravimetric weighing as magnesium pyrophosphate.

THE increased production of complex aluminum alloys has created a difficult problem for the analytical chemist. Present aluminum alloys contain varying percentages of copper, iron, zinc, silicon, manganese, magnesium, chromium, nickel, titanium, and occasional associated impurities such as calcium, lead, tin, and bismuth. Spectrographic methods for the analysis of these complicated alloys have become increasingly popular, but only the larger laboratories have been able to obtain or can afford to purchase spectrographic equipment. The spectrograph does not eliminate the need of accurate chemical methods, since the spectrographer is dependent on the chemical laboratory for an accurate analysis of his spectrographic standards and the frequent checking of his results.

Since magnesium has a most pronounced effect on the physical properties of these alloys, it requires accurate analytical control. Standard basic procedures have been dependent on preliminary separation of the other metals with a final precipitation of the magnesium remaining. These procedures usually make an initial attack of the sample with sodium hydroxide, with addition of hydrogen or sodium peroxide if chromium is present. Samples of high silicon content require a special or modified treatment (*1*). The initial sodium hydroxide attack separates the aluminum as soluble sodium aluminate, leaving the other alloying metals as residuals. These are usually treated with hot, dilute hydrochloric acid to dissolve the magnesium, with a resulting solution of many other metals as well. Experienced analysts have recognized that the magnesium in the residue from the initial caustic attack is not always readily or completely soluble in hydrochloric acid. This may be due to a partial combination of the magnesium as magnesium silicide or other combinations with other alloying metals. Treatment of the residuals with nitric-sulfuric acids is effective in assuring complete solution of the magnesium, but further complicates subsequent separations (*2*).

The "ideal" analytical method indicated a complete solution of the alloy in hydrochloric-nitric-hydrofluoric acids and a direct precipitation of the magnesium. A survey of the literature offered no known reagent specific in a reaction with magnesium in acid solution and in the presence of aluminum and other alloying metals. The very low solubility of magnesium hydroxide indicated promise as an initial separation if the other metals could be held in an alkaline solution as complex compounds. Study and laboratory experiments proved that many of the complexes soluble in alkaline solutions could be formed by the addition of sodium tartrate, but manganese only by the addition of tartaric acid to the acid solution. Efforts to separate nickel quantita-

tively as a cyanide complex were unsuccessful until it was found that pH control was necessary. At a pH of approximately 7, the formation of a nickel cyanide complex soluble in alkaline solution is quantitative. The presence of the tartrates serves the dual purpose of forming soluble metal complexes and acting as a buffer for pH control in the formation of the soluble nickel cyanide complex.

After a final precipitation as the hydroxyquinolate, the magnesium may be determined by the well-known bromate-thiosulfate titration procedure (*3*). Spectrophotometric measurement, however, offers the decided advantages of eliminating the preparation and frequent standardization of volumetric solutions. Several references in the technical literature cover the conversion of magnesium hydroxyquinolate to ferric hydroxyquinolate and subsequent measurement (*4*, *7*, *9*, *10*). The authors find it easier merely to dissolve the magnesium hydroxyquinolate in dilute hydrochloric acid and measure the light transmission or optical density, using a light wave length of 365 mμ. Using a cell having a light path of 1 cm., concentrations up to 6 micrograms per milliliter can be measured. The color is stable for several days and the plot of optical density *vs.* concentration obeys the Beer-Lambert law.

The simpler colorimeters using a tungsten lamp as a light source do not have sufficient sensitivity in the 365-mμ band. A mercury H-4 lamp with a narrow band filter may be used with colorimeters of the two-cell bridge-type circuit such as the Lumetron Model 402E, or the measurement may be made with a spectrophotometer such as the Beckman or the Coleman instrument.

When only an occasional analysis is to be made, the final precipitation of the magnesium may be made as the pyrophosphate with subsequent ignition and weighing. The accuracy of this method is equal to that of the other methods employed.

INSTRUMENTS

Spectrophotometric measurements were made using a Lumetron Model 402E fitted with an H-4 mercury lamp light source, a Sola constant-voltage transformer, a narrow-band 365-mμ glass filter and 1-cm. cells. Also used was a narrow band colorimeter of the authors' own construction having a tungsten lamp light source, a 929 vacuum photocell, and a slide-back potentiometric measuring circuit with two stages of electronic amplification in the null point galvanometer circuit.

REAGENTS

Sodium hydroxide solution, 200 grams dissolved and diluted to 1000 ml. Potassium cyanide solution, 300 grams dissolved and diluted to 1000 ml. Sodium tartrate solution, 300 grams dissolved and diluted to 1000 ml. Tartaric acid solution, 300 grams dissolved and diluted to 1000 ml.

Bromocresol purple indicator, 40 mg. dissolved in 100 ml. of ethanol.

8-Hydroxyquinoline reagent, 20 grams dissolved in 1000 ml. of isopropyl alcohol.

Alcohol-ammonia wash, 500 ml. of isopropyl alcohol, 480 ml. of water, and 20 ml. of ammonia water.

Sodium hydroxide wash, 100 ml. of sodium hydroxide solution diluted to 1000 ml.

Pyridine wash, 15 ml. of pyridine and 40 ml. of ammonia water in 1000 ml. of water.

Ammonium benzoate, 100 grams dissolved and diluted to 1000 ml.

Alizarin Red S, 0.10 gram dissolved in 100 ml. of glacial acetic acid.

Dilute acetic acid, 200 ml. of glacial acetic acid diluted to 1000 ml. Dilute hydrochloric acid, equal volumes of acid and water.

PROCEDURE

Spectrophotometric. Weigh a sample of 0.6 gram of alloys of 0.20 to 2.00% magnesium content, 1.2 grams if the magnesium content is below 0.20%. Place the weighed sample in a 500-ml. Erlenmeyer flask and add 20 ml. of dilute hydrochloric acid, warming, if necessary, to start reaction. When the reaction is complete add 10 ml. of nitric acid and heat to complete solution of the sample. In the presence of silicon in the alloy add hydrofluoric acid in 1- or 2-ml. portions, warming after each addition, until all silicon is dissolved and the solution is clear. Evaporate to drive off most of the free acids but avoid excessive formation of crystals, since these may be difficult to redissolve. A final volume of 10 ml. will usually satisfy these conditions.

Add 30 ml. of hot water, heat to boiling, and dissolve all salts as rapidly as possible. Add 20 ml. of sodium tartrate solution followed by 20 ml. of tartaric acid, boiling the solution vigorously after each addition. Add 8 to 10 drops of bromocresol purple indicator and add sodium hydroxide solution to the appearance of a distinct purple color, carefully avoiding any large excess. Keep at or near boiling during the addition of the sodium hydroxide solution. Add 10 ml. of potassium cyanide solution and boil one minute, then add 30 ml. of sodium hydroxide solution while keeping the solution at a vigorous boil until perfectly clear. Add 100 ml. of hot water slowly and hold just at boiling temperature about 1 or 2 minutes.

Remove the flask from the heat and swirl slowly. After slight cooling, the magnesium hydroxide usually appears as rather fine particles. Digest on a hot plate just below boiling temperature from 3 to 5 minutes. When the precipitate coagulates into rather large clumps, filter through a Whatman No. 30 or No. 40 or equivalent. Wash filter and flask thoroughly with hot 2% sodium hydroxide wash, taking care to remove as much of the aluminum salts as possible. Discard filtrate and washings.

Table I. Determination of Magnesium

	Reported %	Spectrophotometric %	Pyrophosphate %
Bureau of Standards			
No. 85	0.40	0.39	
		0.39	
		0.40	
		0.41	
		0.41	
		Av. 0.40	
No. 85a	1.58	1.57	1.57
		1.58	1.58
		1.57	1.59
		1.59	1.59
		1.58	1.59
		Av. 1.578	1.584
Aluminum Research Institute			
No. 39	0.21 av.	0.20	
		0.21	
		0.22	
		0.22	
		0.23	
		Av. 0.216	
No. 40	0.14 av.	0.14	
		0.13	
		0.13	
		0.15	
		Av. 0.137	
Alcoa spectrographic standards			
SA 104	0.89	0.88	
		0.88	
		0.89	
		0.89	
		0.90	
		Av. 0.888	
SAC 400	0.05	0.05	
		0.05	
		0.055	
		0.06	
		0.04	
		Av. 0.051	
SAC 118	0.50	0.51	
		0.50	
		0.50	
		0.52	
		Av. 0.508	
SSXA 142	1.53	1.53	
		1.53	
		1.53	
		1.51	
		1.54	
		Av. 1.528	

Composition of Samples Used

	85	85a	39	40	104	400	118	142
Cu	4.11	2.48	7.40	0.37	2.00	4.52	2.90	4.10
Sn	0.004	0.001	1.05	0.12	..	..	0.50	..
Pb	0.007	0.002	0.49	0.10	..	..	0.40	..
Zn	0.014	0.02	2.23	0.24	0.50	0.08	2.87	..
Si	0.46	0.11	1.88	5.58	6.79	2.35	2.43	0.12
Mn	0.56	0.66	0.21	0.08	..	0.09	0.21	..
Mg	0.40	1.58	0.21	0.14	0.89	0.05	0.50	1.53
Cr	...	0.23	0.36	0.09	..	..	..	0.19
Ni	...	0.41	0.83	0.10	..	0.17	0.09	2.07
Ti	0.02	0.016	0.11	0.10	..	0.08	0.09	0.11

Retention of some aluminum salts by the filter paper and occlusion by the magnesium hydroxide are unavoidable. Removal of these small traces of aluminum must be made by one of the three following procedures:

(*a*) Dissolve the washed magnesium hydroxide from the filter with 30 ml. of hot dilute acetic acid and wash the paper with hot water. Receive filtrate and washings in the flask in which the original precipitation was made. Hold volume to 60 to 75 ml. Add 10 ml. of ammonium benzoate solution (*6*) and bring just to boiling. Remove from hot plate and allow to stand 2 minutes. Filter on paper of medium texture, wash with warm water, and hold total volume to 100 to 125 ml. Discard paper, and reserve filtrate and washings.

(*b*) Dissolve the washed magnesium hydroxide from the filter with 30 ml. of hot dilute hydrochloric acid and wash paper with hot water, holding volume to 60 to 75 ml. Add 2 drops of bromocresol purple indicator and carefully neutralize with ammonia water to the appearance of a faint purple color. Add 1 ml. of Alizarin Red S solution (*8*) followed by 2 ml. of ammonia water. Heat to temperature of approximately 90° C., remove from heat, and allow to stand until the appearance of a red precipitate. Filter on medium texture paper and wash with warm water. Save the filtrate, and discard filter paper.

(*c*) Follow procedure given under (*b*) through neutralization with ammonia water. Add 2 ml. of pyridine and warm to 45° to 50° C. Remove from hot plate, swirl flask thoroughly several times, and allow to stand a few minutes. The aluminum pyridine complex forms a white gelatinous precipitate. Filter on a paper of usual type and wash with pyridine-ammonia wash water, holding volume to approximately 125 ml.

Remove the small residual traces of aluminum by one of the three procedures given and precipitate the magnesium as the hydroxyquinolate. If procedure (*a*) has been followed, neutralize the solution with ammonia water, using a few drops of bromocresol purple as indicator. To the neutral solution add a minimum of 5 ml. of the 8-hydroxyquinoline reagent plus 1 ml. for 0.1% of magnesium above 0.5% anticipated in the sample. (On samples of low magnesium content the addition of 2 or 3 ml. of tartaric acid solution prior to the neutralization with ammonia water will facilitate the subsequent precipitation of the magnesium hydroxyquinolate.) Add 10 ml. of ammonia water and heat to a vigorous boil. Then hold just below boiling for 5 or 10 minutes or until the precipitate begins to settle. Remove from hot plate and allow to stand an additional 5 minutes.

Filter on Whatman No. 40 or equivalent and wash thoroughly with the alcohol-ammonia wash to remove all excess reagent. Dissolve the magnesium hydroxyquinolate precipitate from the filter with 40 ml. of hot dilute hydrochloric acid and wash paper with hot water. Receive the filtrate and washings in a 200-ml. volumetric flask, Kohlrausch type preferred. Cool thoroughly and dilute to mark. Pipet accurately 20 ml. to a second 200-ml. volumetric flask, add 10 ml. of dilute hydrochloric acid, dilute to mark, and measure transmission or optical density. On alloys of low magnesium content, it is preferable to measure without the second dilution and calculate accordingly.

Preparation of Spectrophotometric Graph. Dissolve 0.5 gram of pure magnesium metal in 50 ml. of dilute hydrochloric acid and dilute to 1 liter. Measure accurately from a buret 30 ml. into a 500-ml. Erlenmeyer flask, dilute to 125 ml., add 20 ml. of 8-hydroxyquinoline reagent and 12 ml. of ammonia water, precipitate, and wash as directed above. Dissolve the precipitate with 40 ml. of hot dilute hydrochloric acid and receive the filtrate and washings in a 500-ml. volumetric flask. Cool and dilute to mark. Measure accurately into 100-ml. volumetric flasks 2-, 4-, 6-, ...20-ml. volumes, add 10 ml. of dilute hydrochloric acid, dilute to mark, and measure transmission or optical density in cells of 1-cm. light path, using light of 365-mμ wave length. These quantities are equivalent to 0.20, 0.40, 0.60.....2.00% magnesium content on the 0.6-gram sample weight specified. Transmission *vs.* concentration plotted on semilog paper should give a straight line. Coleman spectrophotometer paper has been found of a convenient size and arrangement for this and similar graphs.

Bromate-Thiosulfate Method (*3*). Using a sample weight of 0.5 or 1.0 gram according to the magnesium content of the alloy, follow the preceding procedure through the final precipitation and washing of the magnesium hydroxyquinolate. Dissolve the precipitate from the filter with 30 ml. of hot dilute hydrochloric acid, receiving the filtrate and washings in a 500-ml. Erlenmeyer flask. Cool thoroughly, dilute to 125 to 150 ml., add 5 drops of carbon tetrachloride, and with constant swirling of the flask add the standardized potassium bromate solution from a buret until the carbon tetrachloride takes on a faint reddish color. Add 10 ml. of potassium iodide solution and titrate the liberated iodine with standardized sodium thiosulfate to a pale straw color. Add a few milliliters of starch solution and continue the titration to the disappearance of the blue color. Add the last few drops of thiosulfate slowly to ensure reaction with any small amount of iodine held by the carbon tetrachloride. The bromate solution may be standardized by precipitation of known amounts of magnesium as the hydroxyquinolate and titration in the usual manner.

Gravimetric Pyrophosphate Method (*5*). Using 0.5- to 1.0-gram sample weight, precipitate the magnesium hydroxide and wash as directed. Dissolve the precipitate from the filter with 30 ml. of hot, dilute hydrochloric acid and remove the residual aluminum by the use of pyridine as given under procedure (*c*). Receive the filtrate from the pyridine separation in a 400-ml. beaker and make just acid to methyl red indicator, using dilute hydrochloric acid. Add 10 to 20 ml. of saturated diammonium phosphate solution with constant stirring. Add ammonia water slowly with continued stirring until no further precipitation is apparent, then add an excess of ammonia water equivalent in volume to 5% of the original volume. Cover and allow to stand overnight, preferably in an ice box. Use a beaker free of scratches and avoid scratching with the glass stirring rod. Filter and wash with cold water containing 2% ammonia water. Ignite in a weighed porcelain crucible until the precipitate is completely white. After burning off the filter paper, final ignition in a muffle furnace at 1050° to 1150° C. is preferable. Factor = 0.2184.

EXPERIMENTAL CHECKS

Since various metals are used as alloying additions in aluminum alloys or may occur as impurities, synthetic samples of various compositions were prepared. These consisted of the chlorides of nickel, chromium, iron, copper, titanium, calcium, tin, zinc, manganese, aluminum, and lead nitrate, together with known amounts of magnesium as the chloride. These synthetic samples were then analyzed by the spectrophotometric procedure. Magnesium recovered agreed with the known amount present within the limits of experimental error, indicating the noninterference of these metals. Analytical results obtained on two Bureau of Standards samples, two Aluminum Research Institute exchange samples, and four Aluminum Company of America spectrographic standards are reported in Table I. In the certificate furnished with the Alcoa spectrographic standards, the purchaser is warned that: "These standards are prepared and standardized for use in spectrographic analysis by methods similar to those of the Aluminum Company of America. They are not necessarily suitable for use in analytical methods involving the use of other sample forms or procedures."

ACKNOWLEDGMENT

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Determination of Menthol in Peppermint Oil

Acetic Anhydride and Pyridine as Reagent

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THIS study was undertaken to meet the need in a research program on peppermint oil production for simplification and increased accuracy in methods used for the quantitative determination of menthol. The method of Power and Kleber (*7*), generally accepted in the aromatic industry for that compound and "official" in the United States Pharmacopoeia, is both time-consuming and of questioned reliability, since variable results on identical samples of oil have been obtained all too frequently by it in different laboratories.

Brignall (*1*) described a new procedure for the hydroxyl group based on the use of an acetylating mixture consisting of acetic anhydride and *n*-butyl ether. In peppermint oils free menthol determinations by this method have averaged about 3% lower than those done by the method of Power and Kleber. Christensen and Pennington (*2*) worked out a method involving the use of acetyl chloride in determining menthol in peppermint oil. By it, results of menthol determinations in this laboratory agree very well with those obtained by the Brignall procedure but fall distinctly low in comparison with those obtained by the official method. Other methods—viz., one by Delaby, Sabetay, and Brengnot (*3*), one by Freed and Wynne (*4*), and one by Ogg, Porter, and Willits (*5*), each making use of a mixture of pyridine and acetic anhydride—have been proposed for the analysis of primary and secondary alcohols with application of heat. A micromethod, too, has been proposed by Petersen, Hedberg, and Christensen (*6*) using the same reagent without application of heat for determination of the hydroxyl content of pure organic compounds. The authors recently applied this last-mentioned technique on the macro scale for the determination of free menthol in peppermint oils.

ANALYTICAL PROCEDURE

The necessary reagents are: C.P. acetic anhydride, pyridine C.P. and water-free, and sodium hydroxide 0.5 *N* and carbonate-free.

An approximately 0.6-gram sample of oil is introduced into a weighed 7.5-cm. (3-inch) test tube by means of a dropper and its weight again taken accurately to find the weight of sample used. Approximately 0.5 gram of acetic anhydride is introduced into the tube, which is then reweighed. Following the addition of 0.5 cc. of pyridine, the tube is sealed with a cork which previously has been dipped in melted paraffin. The tube is immediately shaken once, set aside at room temperature for 48 hours, then opened and placed in an Erlenmeyer flask. Fifty cubic centimeters of water are added and the solution is titrated with standard sodium hydroxide. Near the end of the titration, flask and contents are heated for a few minutes to ensure complete hydrolysis of the excess acetic anhydride and then titration is carried to the end point of phenolphthalein as shown by persistence of the pink color for 1 minute. A blank titration is carried out at the same time to determine the volume of standard base required to neutralize the acid derived from 1 gram of acetic anhydride. Another sample is weighed and titrated with standard alcoholic sodium hydroxide with phenolphthalein as indicator to determine the amount of free acid in the peppermint oil. The per cent of free menthol is then calculated by the formula

$$\% \text{ free menthol} = \frac{(A \times R - B_1 + B_2)N \times 156.16 \times 100}{W}$$

in which

A = weight in grams of acetic anhydride used
R = ml. of standard base required to neutralize the acid derived from 1 gram of acetic anhydride
B_1 = ml. of standard base required to neutralize the remaining acid
B_2 = ml. of standard base required to neutralize the free acid of the sample
N = normality of standard base
W = weight of sample in milligrams

ANALYTICAL RESULTS

Free menthol was determined on samples of mint oil obtained by steam-distillation and refined by petroleum ether extraction.

Table I shows that an acetylation period of more than 24 hours is necessary and that one of 28 to 32 hours is ample for maximum values.

At least 100% in excess of the theoretical amount of acetylating agent is required (Table II).

The amount of pyridine used may vary within a rather wide range but the ratio of acetic anhydride to pyridine must not be less than 5 to 5 (Table III).

In Table IV each value by the Christensen and Pennington and the official methods is the average of two determinations; each value by the authors' method is the average of two or more determinations. Results by the official method and by the authors' method are in close agreement, while results by the Christensen and Pennington method are from 1 to 4% lower.

Finally the authors' method was used for the recovery of menthol added in varying amounts to 500 mg. of peppermint oil containing 50.6% of free menthol. Recovery values shown in Table V, from single determinations, are considered satisfactory.

Table I. Effect of Variations in Length of Acetylating Period

Acetylation Period *Hours*	Free Menthol Found[a] %
4	44.7
8	46.3
12	46.3
16	47.9
20	48.0
24	47.9
28	50.0
32	50.0
36	49.1
40	49.5
44	50.1
48	49.4

[a] Each value from a single determination.

Table II. Effect of Variations in Amount of Acetylating Agent Used

Acetic Anhydride Used, % of Theoretical Requirement	Free Menthol Found, %
106	38.9
158	47.8
169	47.9
203	49.8
230	50.1
252	50.1
287	49.5

Table III. Effect of Varying Acetic Anhydride–Pyridine Ratio

(Acetylating period 48 hours, room temperature, and at least 100% excess acetic anhydride)

Acetic Anhydride–Pyridine Ratio	Free Menthol Found, %
Without pyridine	11.5
5:1	51.2
5:2	50.1
5:3	51.0
5:4	51.4
5:5	51.1
5:7.5	50.1
5:10	49.9
5:15	49.1
5:20	48.8

Table IV. Results by Different Procedures

Sample	Christensen and Pennington % of menthol	Christensen and Pennington Average deviation	Official or Power and Kleber % of menthol	Official or Power and Kleber Average deviation	Authors % of menthol	Authors Average deviation
A	52.0	0.3	54.0	0.2	53.9	0.1
B	52.6	0.1	53.2	0.5	54.2	0.2
F	51.4	0.2	52.2	0.3	52.4	0.2
G	51.4	0.4	53.1	0.4	53.3	0.1
7	51.2	0.2	52.8	0.6	52.7	0.2
7A	52.1	0.7	54.7	0.1	54.8	0.1
D7	53.1	0.2	56.0	0.6	56.1	0.0
D7A	54.0	0.8	58.0	0.2	57.6	0.1
82	51.0	0.1	51.9	0.4	51.2	0.0

Table V. Recovery of Menthol

Menthol Added *Mg.*	Menthol Actually Present *Mg.*	Menthol Found *Mg.*	Recovery %
60.7	318.7	318.7	100.0
50.7	303.7	304.3	100.2
106.1	363.1	361.5	99.8
102.3	350.3	347.2	99.1
151.6	366.6	363.3	99.1
146.8	375.8	374.6	99.7
184.4	388.4	383.7	98.8
193.0	402.0	395.7	98.5

SUMMARY

From the standpoint of time requirement and reproducibility of results, a very satisfactory procedure has been developed for the determination of free menthol in peppermint oil. It is based upon the utilization of a mixture of acetic anhydride and pyridine. The necessary acetylation period is somewhat longer for menthol than is required by the microprocedure with the same mixture for other alcohols unaccompanied by such a mixture of compounds as that which characterizes peppermint oil. The requirement for acetic anhydride is at least 100% in excess of the theoretical amount. Since the menthol content of peppermint oil varies with the several factors pertaining to production and time of harvesting, it is necessary to add a substantial excess of this reagent. The amount of pyridine used may vary within a rather wide range, but the ratio of acetic anhydride to pyridine must not be greater than 1 to 1. A wider ratio necessitates a longer acetylation period. For peppermint oils containing free acid, the values thus obtained should be corrected.

From this series of experiments it is clear that the Power and Kleber, or official, method for free menthol in peppermint oil is capable of yielding very satisfactory results, if the details of manipulation as given in the U. S. Pharmacopoeia are closely followed. The method devised by the authors, however, involving the use of acetic anhydride and pyridine as acetylating agents, is both more economical of reagents and substantially less time-consuming per sample, particularly when a fairly large number of samples of oil are involved. Samples set aside for acetylation require no attention during the acetylation period. Duplicate results determined by the authors' method check more closely than those by the official method.

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Accelerated Aging Test for Insecticidal Aerosols Containing DDT

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The introduction of DDT into the liquefied-gas aerosol has created the problem of stabilizing the aerosol solution and preventing corrosion of the container. The ease with which hydrochloric acid is liberated from DDT in the presence of some iron salts has made necessary the development of an accelerated aging test for studying the effect of the different aerosol constituents on this reaction. A simple pressure test tube and a method of running a test are described. The rate of decomposition varies greatly, depending mostly on the solvents used. Certain combinations have been developed which appear to be satisfactory.

THE first insecticidal aerosol (*2*) used by our armed forces consisted of a solution of pyrethrum and sesame oil in dichlorodifluoromethane (Freon-12). This aerosol was very effective against mosquitoes, but the addition of DDT was found to increase its toxicity to flies and other insects (*4*). The incorporation of DDT in the aerosol presents several problems, including corrosion of the iron container and formation of tarry materials by decomposition of some of the aerosol constituents.

DDT tends to liberate hydrochloric acid in the presence of some metals and their salts (*1*). Ferric chloride is a particularly good catalyst when the DDT is dissolved in some solvents, but in others very little action takes place. The solvent combination for the DDT aerosol solution is therefore very important. Since DDT is only slightly soluble in Freon-12, some cosolvent must be used to keep even as little as 3% in solution. The choice of a cosolvent must take into consideration its effect on the decomposition of DDT in an iron aerosol bomb. In order to study some of these effects an accelerated aging test was developed. The method of making the test and some results are reported in this paper.

APPARATUS

A simple pressure test tube was devised for these tests. A heavy glass tube was fitted to a tire valve as shown in Figure 1. The construction is described as follows: The frame, 1, was cut from $^1/_2$-inch brass pipe and threaded on the inside of each end with $^3/_8$-inch pipe threads. A standard $^3/_8$-inch brass pipe plug, 2, was drilled, and a tire-valve stem, 3, was soldered in place, about 0.6 cm. (0.25 inch) of the stem extending through the plug. This extension passed through the neoprene washer, 4, and prevented the flow of the rubber, which was under pressure, from closing the hole. The glass test tube, 5, was made from tubing having an inside diameter of 10 mm. and an outside diameter of 15 mm., and was 15 cm. (6 inches) long. It was sealed at one end, cut at right angles and fire-polished at the other end, and then annealed. The last step is very important. A calibration mark was etched on with a small grinding tool at a point where the tube would contain 10 grams of solution. The test tube was held in place against the washer by another $^3/_8$-inch brass pipe plug, 6. A 0.47-cm. ($^3/_{16}$-inch) depression was turned in the plug to accommodate a leather washer, 7, as a pad to protect the glass. The test strip, 8, was usually iron. Its size was $7.5 \times 0.6 \times 0.04$ cm. (3 by 0.25 by $^1/_{64}$ inch), affording twice the surface per unit volume of solution occurring in a 0.45-kg. (1-pound) aerosol bomb.

Different types of valve cores were used. Those having neoprene gaskets and valve seats were best. A special type having a stiff spring, such as is used in the hydraulic system on airplanes, gave the least leakage. An ordinary valve cap was used as an added precaution.

TESTING PROCEDURE

All the apparatus must be carefully cleaned to eliminate the possibility of catalysts that might cause decomposition of DDT. Moisture, hydrochloric acid, or iron salts of any kind must be avoided.

The glass tubes were cleaned in a hot nitric and sulfuric acid bath, rinsed at least 15 times with tap and distilled water, and dried at 110° C. The neoprene washers were boiled in distilled water, washed with pure acetone, and dried at 60° C. The valve stems were cleaned with chloroform and pure acetone and dried at 110° C. The valve cores were washed quickly in chloroform and then in acetone and dried at 60° C.

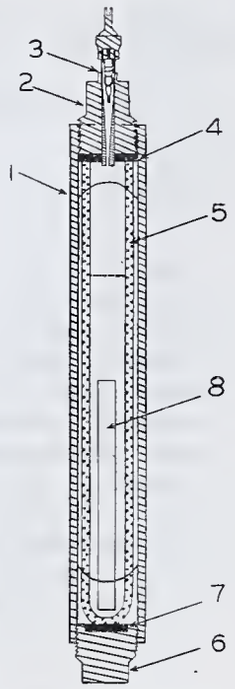

Figure 1. Pressure Test Tube for Testing DDT Aerosols

Most of the nonvolatile materials tested were dried in a vacuum while being heated to 60° C. for at least 30 minutes, and later were stored in a vacuum desiccator. Some were used as received to study the effect of undried materials. Freon-12 was always used as received.

The iron test strips were of the same material used for the manufacture of the aerosol bombs. They were kept under light oil, but some corrosion occurred and had to be removed. The method of cleaning the metal strips affected the results of the tests. Wire brushing, acid treatments, or tumbling with sand was not satisfactory. Apparently certain catalysts were introduced which give erratic results. Fine emery cloth was found to be the best material for cleaning. The method was slow, but uniform results were obtained.

All the nonvolatile materials were weighed into the clean test tubes, the metal strip was added, and the apparatus was assembled. Moderate pressure of the glass tube on the neoprene washer prevented appreciable leakage of Freon for more than 2 months. The Freon was introduced up to the mark all at once from a warm supply to obtain a pressure head. This procedure trapped some air, so that conditions were similar to those in an aerosol bomb, and eliminated condensed moisture that might be carried into the apparatus. The tubes were then placed in an oven at 60° C. They were examined at intervals for corrosion or changes in the system.

The formulas of the aerosol solutions used for these tests are presented in Table I.

RESULTS

At first all tests were terminated at the end of one month The strips of metal were washed with hexane, dried, and weighed. Hexane was used because it does not remove the tars that are often formed. However, the gain in weight which is a combination of the corrosion products and polymerized materials which often occur simultaneously did not appear to be a good criterion of stability. Later the tests were continued until corrosion or tar formation started. The time interval was used as the basis of comparison, and it is on this information that the following statements are based.

The combination tested most frequently (No. 1) contains pyrethrum extract, DDT, cyclohexanone, and lubricating oil dissolved in Freon-12. The stability of the solution depends first on the method of manufacturing the pyrethrum extract. Four kinds of pyrethrum extract were tried in this formula, and it was learned that the extracts having the most naturally occurring inhibitors are best. Various types of lubricating oils also had a considerable effect. Aromatic base oils seemed to be the best, and some of the oils tested may have contained inhibitors. Three grades of DDT were used in combination 1. A purified grade of DDT is generally better than a technical grade; some technical grades are better than others. Cyclohexanone appears to reduce the stability of the mixture. Only freshly distilled anhydrous material can be used and this is variable. This mixture usually breaks down in less than one month at 60° C.

The thiocyanate insecticides (Nos. 3 and 4) are not so stable as pyrethrum extracts in a DDT aerosol solution. The mixture of pyrethrum and sesame oil (No. 5) without DDT is stable over very long periods, and has never caused corrosion of iron nor tar formation. Synergist 312 (No. 6) has shown poor stability in two tests, causing tar formation in 26 days.

A combination such as No. 12 can be handled most easily, and gives very good results in these tests. It contains pyrethrum extract, DDT, and an aromatic petroleum fraction in Freon-12. Various proportions have been tested and none has broken down before 50 days, while some have run more than 100 days. This aromatic petroleum cosolvent, a by-product in the manufacture of high-octane gasoline, consists mostly of polymers of alkylated naphthalenes and anthracenes, but the exact composition is not known.

Two tests with isophorone (No. 7) show that it forms a fairly stable solution, causing tar formation in 52 days. Acetone (No. 8) in combination with DDT in methyl chloride causes definite corrosion of iron, but no tar. Methylene chloride (No. 9) is a very good diluent for Freon-12 (*3*) and a good cosolvent to keep DDT in solution, but is not so stable in the aerosol mixture as would be desired. The period before decomposition was 44 days.

Propylene oxide (No. 2) is both an excellent solvent for DDT and a stabilizer. Its ability to accept nascent hydrochloric acid appears to stabilize the DDT aerosol solution. No corrosion nor tar formation has occurred in tests running as long as 150 days. The combination (No. 2) of the aromatic petroleum fraction and propylene oxide appears to give the most stable DDT aerosol solution. Propylene oxide has the disadvantage of being highly flammable and slightly toxic, so that considerably more care must be used in manufacturing the aerosol bomb. It is not flammable when mixed with Freon in the proportions needed.

Dioleyl malate (No. 10) appears to give added stability to the solution in some cases. Degras fatty acids (No. 11) give no protection.

Freon-12 appears to give a more stable solution than methyl chloride. Not many tests have been made on methyl chloride, since it is not used in the military aerosol bomb.

Table I. Composition of Basic Formulas Tested

	Per Cent by Weight in Following Formulas											
	1	2	3	4	5	6	7	8	9	10	11	12
Constituent												
Insecticides												
Pyrethrum extract (20%)	2	2	2	2	2	2	2	..	2	2	2	2
DDT	3	3	3	3	..	3	3	3	5	3	2	3
Lethane[a]	..	..	..	5	..	..	..	..	..	..	...	..
Thanite[b]	..	..	5	..	..	..	..	..	..	..	...	..
Synergists												
Sesame oil	..	..	..	..	8	..	..	..	..	...	...	..
Synergist 312[c]	..	..	..	..	..	3	..	..	..	...	...	..
Solvents												
Methylene chloride	..	..	..	..	..	..	..	..	30	..	...	..
APS-202[d]	..	7	..	..	..	12	..	..	..	...	8	12
Cyclohexanone	5	..	5	5	..	..	..	5	..	5	...	..
Lubricating oil (No. 30)	5	..	..	..	..	..	5	..	5	5	...	..
Acetone	..	..	..	..	..	..	..	35	..	...	...	..
Isophorone	..	..	..	..	..	..	5	..	..	...	...	..
Velsicol[d]	..	..	..	..	..	..	..	5	..	...	...	..
Inhibitors												
Dioleyl malate	..	..	..	..	..	..	..	..	..	0.03	...	..
Degras fatty acids	..	..	..	..	..	..	..	..	..	...	0.03	..
Propylene oxide	..	5	..	..	..	..	..	..	..	...	...	..
Liquefied gases												
Freon-12[e]	85	83	85	85	90	80	85	..	60	85—	88—	83
Methyl chloride	..	..	..	..	..	..	..	50	..	...	...	..

[a] β-Butoxy-β′-thiocyanodiethyl ether.
[b] Bornyl and fenchyl thiocyanoacetates mixture.
[c] 3-Hexyl-5-piperonyl-2-cyclohexen-1-one and 6-carboethoxy-3-hexyl-5-piperonyl-2-cyclohexen-1-one mixture.
[d] Alkylated naphthalenes from petroleum.
[e] Dichlorodifluoromethane.

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Determination of Traces of Acetylene in Liquid Oxygen in Rectifying Columns

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A modified method for determining very small amounts of acetylene in liquid oxygen from air-rectifying columns is presented. As acetylene is dangerous in these columns and will sometimes explode violently, studies were made of the acetylene content when (1) different lubricating oils are used, (2) calcium carbide is stored near an oxygen plant, (3) liquid oxygen is periodically drained from the columns and discarded, (4) the air intake location is changed, and (5) an acetylene plant is located in close proximity to an air-reduction plant.

IT IS generally believed that explosions in the pure oxygen pot of rectifying columns producing oxygen from liquid air are attributable to acetylene. Acetylene may be present in the crude air or may be formed from the breakdown of the lubricating oil in the air compressor. As oxygen boils at −183° C. and acetylene freezes at −81° C., nearly all the acetylene entering the column will remain there.

However, it is distinctly possible that other hydrocarbon materials can produce explosions in rectifying columns, because practically all hydrocarbon materials will burn with oxygen and many will explode with liquid oxygen. Some detonator is required to set the explosion off, although acetylene and other materials may detonate spontaneously. All this goes to show the great danger in the presence and accumulation of hydrocarbon materials in liquid oxygen condensers.

From the standpoint of safe operation, a method for determining traces of acetylene in liquid oxygen was needed. Since many oxygen plants do not have a trained chemist available for analytical work, it was desirable that the method developed be one which could be used by plant operators.

LITERATURE REVIEW

Practically nothing on the subject has been published in this country, although many Russian papers deal with this work, and some German articles and several English papers have been reported.

Pollitzer (*4*) states that about 80% of the acetylene in the air will remain in the apparatus. He cites as an example that, in a plant making 100 cubic meters of oxygen per hour, using air that contains 5 parts per million of acetylene, about 1 kg. of acetylene will enter the apparatus in the course of 14 days and about 0.8 kg. will remain. The exact quantity of acetylene needed to produce an explosion is not accurately known. A few grams suffice, under certain conditions, to produce a strong local explosion.

Burbo (*1*) discusses the dangerous deposition of solid acetylene in the condensers of the oxygen column. He gives the vapor pressure of solid acetylene as from 89° to 105° Kelvin. If all oxygen is continuously evaporated from the main condenser, a maximum of 0.184 cc. of acetylene is evaporated per cubic meter of oxygen, corresponding to a maximum concentration of 0.037 p.p.m. of acetylene in the incoming air, in excess of which solid acetylene will accumulate.

Coulson-Smith and Seyfang (*2*) describe a colorimetric method based on the color of copper acetylide in colloidal solution (Ilosvay's solution). A series of mixtures of air and acetylene was prepared and 100 ml. of each were passed through ammoniacal cuprous chloride solutions. The resulting cuprous acetylide solutions were then matched by running measured amounts of a standard iron solution into a 5% potassium thiocyanate solution. One of the present authors checked these results, using a water solution of acetylene made from carbide, purifying it to remove phosphine and hydrogen sulfide, and passing the gas through distilled water. The acetylene content was determined by adding 10 ml. of 0.01 *N* silver nitrate, filtering off the silver acetylide, and back-titrating the remaining silver nitrate with 0.01 *N* potassium thiocyanate. The value obtained was 0.14 ml. of acetylene per ml. of water. Next, various amounts of the acetylene water were run into ammoniacal cuprous chloride solution (described below) and the color was obtained by the iron solution and potassium thiosulfate of Coulson-Smith and Seyfang. Excellent checks were obtained (see Table I).

When determining acetylene in liquid oxygen, the gasified oxygen cannot be bubbled directly through the Ilosvay solution (ammoniacal cuprous chloride), since it will oxidize the colorless cuprous solution to a blue cupric compound and hence will interfere with the color comparison. Obviously the sample of liquid oxygen must first be gasified and the acetylene frozen out.

Oks (*3*) describes a condenser for such a purpose. He also used a large copper funnel of 5000-ml. capacity, tapering down to a neck of 500-ml. capacity. The sample of liquid oxygen was boiled down to 500 ml. A stopper was then inserted in the neck, water poured in to make an ice seal, and the balance of the gasified oxygen run through the condenser.

The authors have modified the methods of Coulson-Smith and Seyfang and of Oks (the best Russian work), for the determination of acetylene in liquid oxygen.

EXPERIMENTAL WORK

Work of Coulson-Smith and Seyfang (*2*) was checked (Table I).

The authors used the iron solution as a standard in their first tests, but calibrated a Corning glass filter No. 348 (Table II) for a permanent standard. The solution used was prepared as follows:

CUPROUS CHLORIDE. Twelve grams of cuprous chloride were dissolved in 300 ml. of distilled water and 135 ml. of concentrated ammonium hydroxide were added, and then stirred. The solution was transferred to a bottle in which some copper wire had been placed.

HYDROXYLAMINE HYDROCHLORIDE SOLUTION. Seventy grams of hydroxylamine hydrochloride were dissolved in 300 ml. of warm water and placed in a stoppered bottle.

STARCH SOLUTION. Soluble starch (2.5 grams) was mixed in 5 ml. of cold water poured into 300 ml. of boiling water. This was boiled for 2 minutes, cooled, and diluted to 500 ml.

To 20 ml. of the cuprous chloride were added 30 ml. of hydroxylamine hydrochloride and 20 ml. of starch solution. This was placed in a 100-ml. Nessler tube and 30 ml. of distilled water were added, followed by the acetylene water. The starch was used in order to keep the cuprous acetylide precipitate in a colloidal state for easier color comparison.

Another method of determining the acetylene is to bubble the gasified sample through an ammoniacal silver nitrate solution, filter the silver acetylide precipitate off, dissolve it in nitric acid, and titrate the silver with potassium thiocyanate. Oks (*3*) shows that the results obtained by this method are about five times as low as those obtained with the ammoniacal cuprous chloride solution.

The authors decided that the ammoniacal cuprous chloride method was the better one for their purpose.

Table I. Determination of Acetylene

C_2H_2 Water	Ferric Alum to Match Color (0.35-Ml. Blank)	Equivalent Ferric Alum	C_2H_2 Content of Water	
			Coulsen-Smith and Seyfang	Eddy
Ml.	*Ml.*	*Ml.*	*Ml.*	*Ml.*
0.1	0.50	0.15	0.015	0.014
0.2	0.62	0.27	0.027	0.028
0.3	0.74	0.39	0.039	0.042
0.4	0.87	0.52	0.052	0.056
0.5	1.05	0.70	0.070	0.070
0.6	1.22	0.87	0.087	0.084
0.7	1.37	1.02	0.102	0.098
0.8	1.50	1.15	0.115	0.112

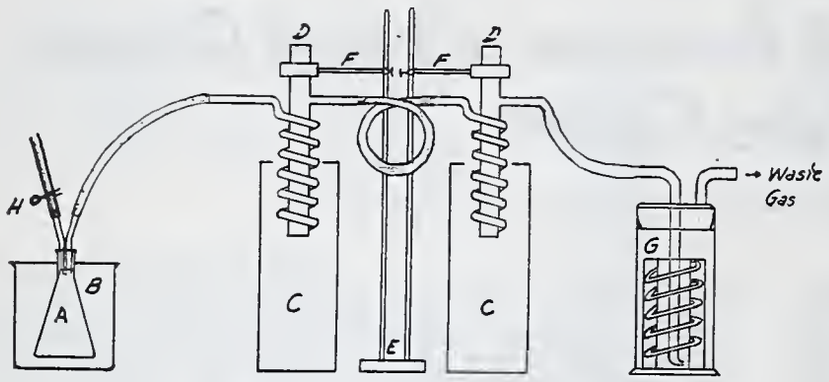

Figure 1. Apparatus for Acetylene Test

A. Erlenmeyer flask
B. Pyrex beaker
C. Pyrex thermos bottles
D. Oks condensers
E. Double ring stand
F. Buret clamps
G. Milligan absorption bulb
H. Pinchclamp

The funnel described by Oks (*3*) was found too cumbersome in use. The ice plug blew out on the trial runs, thus making a better arrangement desirable.

Finally, an Erlenmeyer flask was placed in a 100-ml. beaker and packed with rock wool. The sample of liquid oxygen was placed in the flask, stoppered with a two-hole rubber stopper, and the gasified oxygen passed through two of the Oks condensers, which were immersed in liquid oxygen (see Figure 1 for details of this equipment). A 250-ml. Erlenmeyer flask was used when testing samples above 0.7 p.p.m. A larger sample was taken when dealing with lower concentrations.

After all the oxygen had vaporized (evidenced by melting of the frost on the rubber tubing), the first condenser was lifted out of its cooling liquid and examined for recondensed oxygen. Oxygen recondenses if the condensers are inserted too far into the liquid oxygen. If any was present, it was slowly vaporized off by lifting the condenser out of the liquid, but without removing the vacuum flask, since evaporation would then be too fast and the acetylene would be carried off. This was repeated for the second condenser.

Next the absorption bulb, filled with 150 ml. of Ilosvay solution, was connected to the last condenser; the vacuum flasks were removed, and a nitrogen supply was connected to the Erlenmeyer flask at low pressure. The pinchclamp was removed and nitrogen was bubbled through for a half hour.

When the first part of the gas was passed through, the solution turned blue owing to the oxygen present, but this soon disappeared as the excess hydroxylamine hydrochloride reduced it to the colorless form. A pink to red color was imparted to the solution by the acetylene, depending upon the amount present.

The absorption bulb was removed and the color compared with the comparator part of the colorimeter. A 100-ml. tube of distilled water was placed over the glass to simulate the conditions of the unknown. The glass was calibrated by means of the ferric alum solution. Since only 100 ml. of the 150 ml. of solution from the absorber were used, when 100 ml. of solution matched the glass filter, the ferric alum equivalent of the whole solution would be 150/100 of 0.94 or 1.43 ml., equivalent to 0.143 ml. of acetylene. Since 250 ml. of liquid oxygen are equal to 200,000 ml. of gaseous oxygen, the acetylene content on the gas basis would be 0.7 p.p.m.

Because the depth of color of cuprous acetylide solutions bears a linear relationship to the concentration of acetylene, a deeply colored solution could have been halved or quartered and made up to the original volume and the acetylene multiplied by 2 or 4.

Oks (*3*) uses only one condenser for his work. He claims complete freezing out of the acetylene while maintaining a temperature of −160° C. inside the condenser. The authors found that a sample showed 2.0 p.p.m. of acetylene with one condenser, and 3.3 p.p.m. with two condensers. Another sample showed 2.1 p.p.m. of acetylene with two condensers and only 2.5 p.p.m. with four condensers. The authors concluded that two condensers would be sufficient for all practical purposes. That not all the acetylene is frozen out is due to the vapor pressure of the acetylene even at extremely low temperatures. In addition, the Oks condensers require stopcocks; these are not necessary in the authors' technique.

Weaver (*8*) states that small amounts of carbon dioxide did not affect the colorimetric determination of acetylene, but larger amounts had the same effect as the introduction of a strong electrolyte into the solution. The color of the colloid produced by acetylene carried over in a stream of pure carbon dioxide was too brown to permit any accurate comparison.

A brown scum frequently appeared on the top of the solution in the absorber, but was never strong enough to ruin the test. Apparently this was due to carbon dioxide which would freeze out with the acetylene.

Riese (*5*) states that the stability of Ilosvay's solution is limited, and recommends that the work always be done with freshly prepared solutions. By making up the three components of the Ilosvay solution separately and mixing only when ready for use, as the authors have done, there is always a fresh solution available.

Riese further notes that addition of more ammonia is not to be recommended, since excess ammonia causes the color of the colloid to become more orange in addition to increasing the sensitivity of the solution toward oxygen.

After the authors had used the above method in their laboratory for some time, they supplied the operators in various plants with the equipment shown in Figures 1 and 2, as well as suitable graduates, beakers, and reagent bottles. Since all oxygen plants do not have suitable balances for weighing the solid chemicals, the cuprous chloride was weighed out in 12-gram packages wrapped in paper, and kept in a wide-mouthed bottle. The hydroxylamine hydrochloride was also repackaged into small bottles, so that each bottle was just sufficient for one batch of solution.

A set of instructions for using the equipment and making the test was drawn up and explained to the operators and two or three tests were run through with those expected to continue making the tests. They found no difficulty in running the tests as efficiently as an experienced chemist would have done. During the past year, the authors have been running tests on seven different columns at three different locations and have studied the effects of various factors. The results of tests are summarized below.

Effect of Different Brands of Lubricating Oils. Comparative tests were run, using three different grades of lubricating oil in compressors; a noticeable difference was found in the amount of acetylene accumulated in the column with the different brands of oil.

Effect of Carbide Storage in Air Plants. At the time that daily tests on columns were being run, a broken drum of carbide

Table II. Calibration of No. 348 Corning Filter

(250-cc. sample of liquid oxygen)

Unknown	C_2H_2	Unknown	C_2H_2
Ml.	*P.p.m.*	*Ml.*	*P.p.m.*
100	0.70	50	1.41
90	0.78	40	1.76
80	0.88	30	2.35
70	1.00	20	3.54
60	1.25	10	7.10

Table III. Effect of Carbide Spilled near Plant

Date (1944)	C_2H_2 by Volume Column 1	Column 2
	P.p.m.	*P.p.m.*
1/24	0.25	0.35
1/26	0.75	1.10
1/27[a]	2.60	2.00
1/28	1.50	2.10
1/31	0.90	1.30
2/1	0.60	1.20

[a] Carbide spilled on platform previous night.

was returned by a customer and left on the loading platform overnight. Some of it was spilled on the platform and during the night it rained. Tests on the columns the following day showed its presence, as evidenced by Table III. Column 1 showed the largest increase in these tests, as its air intake was closest to the rising acetylene over the dome of the roof.

Effect of Draining Liquid Oxygen. Strizhevskiĭ (7) states that no method for the safe application of the rectifying apparatus has been found. One method for preventing explosions consists in removal of the liquid from the condenser in which considerable amounts of acetylene accumulate. For this purpose, it is necessary to conduct systematic analyses of the liquid oxygen in the condenser for the presence of acetylene.

Previous to the acetylene testing the authors had been draining and discarding 1 quart of liquid oxygen every 2 hours in the belief that this would reduce the acetylene accumulation in the columns. As daily tests on two 100-meter columns at the same plant gave practically the same results, draining column 2 was discontinued while draining column 1 was continued. The results are tabulated in Table IV.

It was concluded that draining liquid periodically from the main kettle had no effect on the accumulation of acetylene in these columns.

Effect of Moving Air Intake. One column ran much higher in acetylene content than the other columns. Its air intake was in a considerably less advantageous position than the others, in that it was lower and more vulnerable to strange ground currents. Raising the intake obviated this difficulty to a great extent. The average acetylene concentration for the 6 days prior to raising the air intake was 0.8 p.p.m., and for the 9 days after raising the air intake was 0.2 p.p.m.

Effect of Acetylene Plant in the Neighborhood. Tests were made on an oxygen plant located near an acetylene plant which operates intermittently. On days when the acetylene plant was in operation, the tests were noticeably higher than

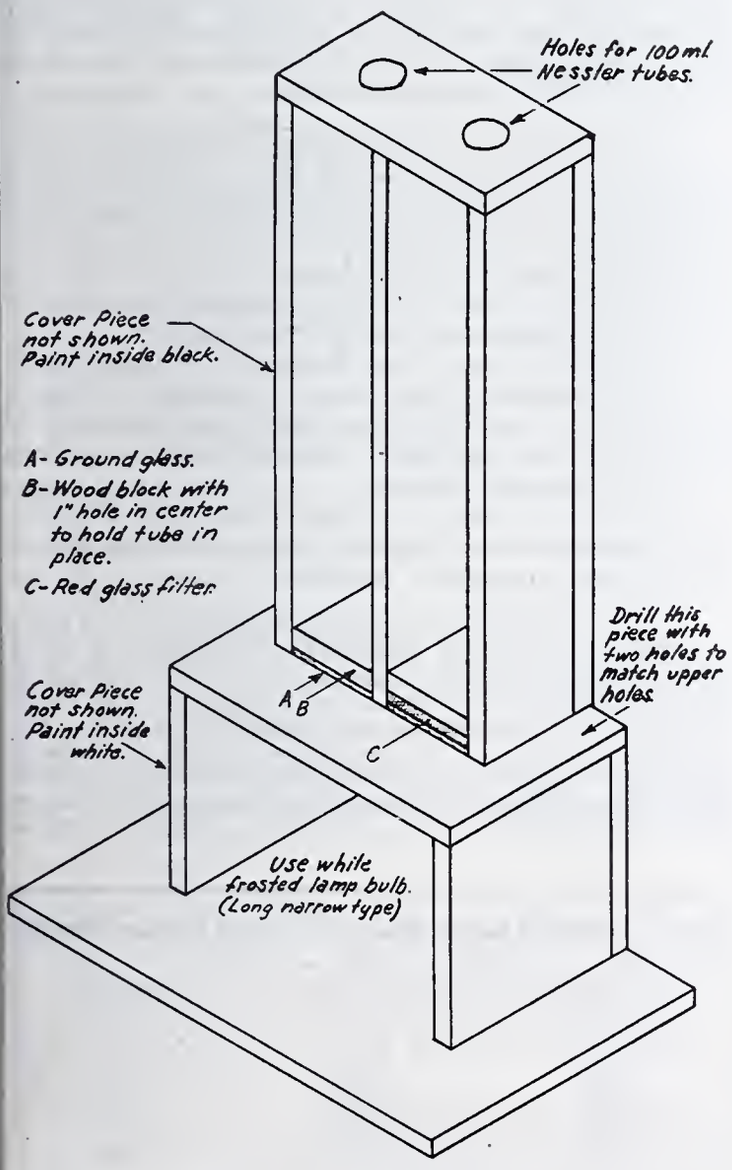

Figure 2. Colorimeter Design

Table IV. Effect of Draining Liquid

Date (1944)	Column 1 Days run since defrosting	Column 1 Test C_2H_2 P.p.m.	Column 2 Days run since defrosting	Column 2 Test C_2H_2 P.p.m.
2/10	19	1.8	1	2.5
2/11	20	0.7	2	0.4
2/12	21	0.2	3	0.0
2/14	1	..	5	0.4
2/15	2	0.5	6	0.5
2/16	3	1.0	7	1.0
2/17	4	1.0	8	0.7
2/18	5	0.5	9	0.5
2/19	6	0.5	10	0.5
2/21	8	0.5	12	0.5
2/22	9	0.6	13	0.5
2/23	10	0.3	14	0.3
2/25	12	0.7	16	0.7
2/26	..	..	17	0.6
2/27	14	0.2	18	0.7
2/28	15	0.7	19	0.7
2/29	16	0.7	20	0.0

Table V. Effect of Acetylene Plant Operating in Neighborhood

Date (1944)	Time	Days Run since Defrosting	C_2H_2, P.P.M.
3/8	11:00 A.M.	3	2.65
3/8	2:00 P.M.	3	0.87[a]
3/8	4:20 P.M.	3	0.70[a]
3/9	6:00 A.M.	4	3.40
3/9	10:35 A.M.	4	1.30[a]
3/9	12:15 P.M.	4	0.70[a]
3/9	2:30 P.M.	4	0.70[a]
3/10	8:00 A.M.	5	2.74
3/10	11:00 A.M.	5	1.25
3/10	2:00 P.M.	5	0.98[b]
3/11	8:00 A.M.	6	5.85[b]
3/11	11:00 A.M.	6	1.35
3/11	12:00 M.	6	1.05[a]
3/11	2:00 P.M.	6	0.75[a]
3/11	6:00 P.M.	6	0.70[a]
3/11	8:00 P.M.	6	0.73[a]
3/11	11:50 P.M.	6	0.95[a]
3/12	8:00 A.M.	7	0.70[a]
3/12	10:00 A.M.	7	0.70[a]
3/12	2:00 P.M.	7	0.70[a]
3/12	6:00 P.M.	7	0.70[a]
3/12	8:30 P.M.	7	0.70[a]
3/12	11:40 P.M.	7	0.60[a]
3/13	10:35 A.M.	8	1.12[c]
3/13	12:45 P.M.	8	0.94
3/13	3:00 P.M.	8	0.90
3/14	2:00 A.M.	9	0.88[a]
3/14	7:00 A.M.	9	0.75[a]
3/14	9:00 A.M.	9	0.70[a]

[a] Acetylene plant not operating.
[b] Liquid drawn every 2 hours between these two tests.
[c] Strong wind blowing away from air intake.

when it was shut down. The prevailing winds blow from the air plant to the acetylene plant and would tend to blow the acetylene away from the plant. There was considerable correlation between wind velocity and the amount of acetylene in the oxygen when the acetylene plant was operating. Extremely high concentrations of acetylene were not found at any time and, although the column ran continuously, it would clean itself out when the acetylene plant was idle.

This self-cleaning of columns is referred to by Burbo (*1*) and was also noted in the case of the carbide being spilled on the loading platform.

Table V gives sample date from this location. Even when the acetylene plant was operating all day, the first test was invariably higher than the following ones. To check whether or not this was due to the drawing off of liquid, a pint of liquid was drawn off every 2 hours between the last test of March 10 and the first test of March 11. This test was the highest of any reported. Therefore, it is apparent that the draining of liquid does more harm than good.

One explanation for gradual decline of the tests during the day, while the acetylene plant is operating, is that the prevailing winds rise during the day and may blow the acetylene away from the oxygen intake.

CONCLUSIONS

This method involves the use of a glass filter as a permanent color standard. It has been simplified to a point where analyses can be made by nontechnically trained operators. This method is,

under any circumstances, accurate enough to give good comparisons; tests run on seven different columns at three different locations have indicated changes which have led to safer operating conditions.

For more exact work where the absolute value of acetylene is desired, the rubber stoppers and tubing may be replaced with glass and cold filters as described by Shepherd (*6*) used.

ACKNOWLEDGMENTS

The authors wish to acknowledge the assistance of George R. West and the staff of the Stuart Oxygen Co. Research Department who did some of the preliminary work on the method; and of Merle Randall who gave many valuable suggestions. They also wish to acknowledge the work of the operators in the various plants who ran most of the actual tests after the method had been developed.

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Determination of Cuprous Chloride

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DURING recent hydrolysis studies (*5*) it became necessary to determine accurately the purity of solid cuprous chloride and the cuprous chloride content of dilute solutions. As direct titration of cuprous chloride did not give satisfactory results, the indirect method of the AMERICAN CHEMICAL SOCIETY Committee on Analytical Reagents (*1*) was tried. This method lacked both the precision and accuracy required for the analysis of samples of essentially pure solid cuprous chloride. This paper calls attention to the defects of the A.C.S. method and proposes analytical procedures which are more satisfactory for both solid cuprous chloride and its dilute solutions.

EXPERIMENTAL

REAGENTS. Merck reagent grade cuprous chloride was carefully purified by the usual methods (*6*). Determination of chloride ion (by the Volhard method) and of total copper by electrodeposition (after conversion to cupric sulfate) indicated a composition of 99.8% cuprous chloride and 0.2% cupric chloride. The dry cuprous chloride was placed in small glass-stoppered vials and stored in a desiccator over magnesium perchlorate. Cuprous chloride thus prepared and stored showed no change in composition over a period of several months.

All other chemicals used were of reagent grade.

A.C.S. METHOD. Following is the method which is approved by the A.C.S. Committee on Analytical Reagents (*1*) for the determination of cuprous chloride in a solid sample:

Dissolve 0.5 gram in the cold in 25 ml. of ferric ammonium sulfate solution, made by dissolving 10 grams of ferric ammonium sulfate in 100 ml. of dilute hydrochloric acid (1 + 1), add 5 ml. of phosphoric acid, dilute with 200 ml. of water, and titrate with permanganate, correcting for blank on reagents.

Careful application of this method consistently gave low values and poor precision when the sample was essentially 100% cuprous chloride. The degree of error is related to the sample size (Table I).

On the basis of the oxidation-reduction potentials involved, it is expected that complete oxidation of cuprous copper will occur, if a slight excess of ferric iron is present (*4*). Actually, the determination of cuprous chloride by this method is the same as a determination of the equivalent amount of ferrous iron in the presence of the excess ferric iron added and an amount of cupric chloride equivalent to the cuprous chloride of the sample. Factors which would influence the titration are cupric ion, chloride ion, and ferric ion concentration and the nature of the acid medium and of the oxidizing agents.

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A study of some of these factors was made by the titration with 0.1 *N* potassium permanganate of 25-ml. samples of a standard solution of ferrous chloride in 1 + 1 hydrochloric acid, in the presence of added cupric chloride and ferric ammonium sulfate as indicated in Table II. Two hundred milliliters of water and 5 ml. of phosphoric acid were used as in the A.C.S. procedure. The added amounts of cupric chloride are the oxidation equivalents of the recorded amounts of cuprous chloride. The indicated amounts of cupric chloride and of ferric alum were added by measuring appropriate volumes of their standard solutions. The amount of ferrous chloride found by titration, in the absence of added cupric chloride or ferric alum, is taken as 100% to simplify comparison.

USE OF OXIDIZING AGENTS OTHER THAN POTASSIUM PERMANGANATE. A comparison of the effect of the presence of cupric chloride on the titration of ferrous chloride using oxidizing agents other than potassium permanganate was made. The data in Table III were obtained by the titration of 25-ml. samples of a 1% sulfuric acid solution of ferrous ammonium sulfate by the indicated oxidizing agents, using standard procedures.

The effect of the acid used to dissolve the ferric ammonium sulfate when cuprous chloride is analyzed was also determined. A comparison of the different acids and of the three oxidizing agents used is given in Table IV (analytical methods are given below under Recommendations).

To determine the effect of dissolved oxygen on the determination of cuprous chloride the ferric ammonium sulfate solution used was made "oxygen-free" as follows:

More than the required amount of distilled water was boiled for 30 minutes and cooled quickly while being swept by carbon dioxide. Part of this water was poured into a volumetric flask which had been swept with carbon dioxide, and the correct amounts of hydrochloric acid and of ferric ammonium sulfate were added. Carbon dioxide was bubbled through until solution was complete and water had been added to give the correct total volume. The use of 25 ml. of this "oxygen-free" *N* hydrochloric acid solution of ferric ammonium sulfate followed by

Table I. Effect of Sample Size in Analysis of Cuprous Chloride

(Actual % CuCl = 99.8)

Weight of Sample	Cuprous Chloride Found	Precision
Gram	%	± %
0.50	97.5	1.0
0.45	98.6	0.2
0.40	98.8	0.2
0.35	98.9	0.1
0.30	98.9	0.1
0.25	98.9	0.1
0.20	98.9	0.1

Table II. Effect of Cupric Chloride and Ferric Alum on Titration of Ferrous Chloride by Permanganate

Run	CuCl Equivalent to Added $CuCl_2$	Ferric Alum Added	Ferrous Chloride Found
	Gram	*Gram*	%
A	0.00	0.0	100.0
B	0.25	0.0	94.7
C	0.25	1.0	98.7
D	0.50	0.0	93.9
E	0.50	1.0	98.7
F	1.00	0.0	93.4
G	1.00	1.0	98.2

Table III. Effect of Cupric Chloride upon Titration of a 1% Sulfuric Acid Solution of Ferrous Ammonium Sulfate

CuCl Equivalent to Added $CuCl_2$	Ferrous Chloride Found		
	$KMnO_4$	$K_2Cr_2O_7$	$Ce(SO_4)_2$
Gram	%	%	%
0.00	100.0	100.0	100.0
0.25	96.9	99.7	99.8
0.50	95.3	99.5	99.8

itration with potassium dichromate gave 99.5% cuprous chloride as compared with 99.2% of a similar solution, made in ontact with air was used.

DISCUSSION

The poor results obtained in the analysis of 0.5-gram samples f cuprous chloride by the A.C.S. method may be caused by an nsufficient amount of ferric ammonium sulfate solution for the xidation of the cuprous copper. From the specification "25 l. of ferric ammonium sulfate solution, made by dissolving 10 rams of ferric ammonium sulfate in 100 ml. of dilute hydrohloric acid" it is assumed that the intention is to use 2.5 grams f ferric ammonium sulfate. This would allow a slight excess of xidizing agent, since 0.5 gram of cuprous chloride is equivalent o 2.44 grams of ferric alum. However, the addition of 100 grams f ferric ammonium sulfate to 1000 ml. of 1 + 1 hydrochloric cid, in a constant-temperature bath at 30° resulted in 1060 ml. f solution, containing only 2.36 grams of ferric ammonium sulate per 25 ml. Thus, part of the cuprous chloride is oxidized by rric iron, part by permanganate, and an indefinite amount by tmospheric and dissolved oxygen (*3*), the latter amount being roportional to time and to the amount of agitation (*2*). For .5-gram samples of essentially pure cuprous chloride, the permanganate concentration should be slightly more than 0.1 *N*, nce 0.5 gram of cuprous chloride is equivalent to more than 50 l. of 0.1 *N* solution. Table II indicates that the adverse effects f cupric ion are partially, but not entirely, offset by the presence f the ferric alum.

Comparison of runs A, B, and D of Table II with the data in able III indicates that the effect of cupric chloride upon the ermanganate titration of ferrous iron is lessened by decreased hloride ion concentration and that the effect of cupric chloride is ss when potassium dichromate or ceric ammonium sulfate is sed.

RECOMMENDATIONS

The following three methods for the determination of the uprous chloride content of solid cuprous chloride have all proved uperior to that recommended by the A.C.S. Committee on nalytical Reagents:

Potassium Permanganate. Weigh 0.3 gram of cuprous loride into a dry 500-ml. Erlenmeyer flask, add 25 ml. of ferric mmonium sulfate solution, made by dissolving 10 grams of rric ammonium sulfate in sufficient 3 *M* sulfuric acid to make 0 ml., and swirl gently until dissolved. Add 200 ml. of water d 5 ml. of phosphoric acid, and titrate with 0.1 *N* permanate to the first pink color which persists for 15 seconds. A ank must be run on the reagents.

Potassium Dichromate. Weigh 0.3 gram of cuprous chloride to a dry 500-ml. Erlenmeyer flask, add 25 ml. of ferric amonium sulfate solution, made by dissolving 10 grams of ferric mmonium sulfate in sufficient *N* hydrochloric acid to make 100 ml., and swirl gently until dissolved. Add 300 ml. of a solution containing 80 ml. of sulfuric acid and 25 ml. of phosphoric acid per liter, add 5 drops of 0.2% barium diphenylaminesulfonate indicator solution, and titrate with 0.1 *N* dichromate to the first permanent purplish tinge. A blank must be run on the reagents.

Ceric Ammonium Sulfate. Weigh 0.3 gram of cuprous chloride into a dry 500-ml. Erlenmeyer flask, add 25 ml. of ferric ammonium sulfate solution, made by dissolving 10 grams of ferric ammonium sulfate in sufficient 3 *M* sulfuric acid to make 100 ml., and swirl gently until dissolved. Add one drop of ferrous-phenanthroline indicator solution and titrate with 0.1 *N* ceric ammonium sulfate solution (made up in 0.5 *M* sulfuric acid solution). A blank must be run on the reagents.

For determination of the cuprous copper content of dilute solutions the following procedure is recommended:

Pipet 25 ml. of the cuprous copper solution into 25 ml. of ferric ammonium sulfate solution, made by dissolving 33 grams of ferric ammonium sulfate in sufficient 3 *M* sulfuric acid to make 1000 ml. Add 250 ml. of water and 1 drop of ferrous-phenanthroline complex indicator solution, and titrate with 0.1 *N* ceric ammonium sulfate solution (made up in 0.5 *M* sulfuric acid). A blank must be run on the reagents.

This quantity of ferric iron is sufficient for solutions containing up to 0.6% cuprous chloride.

For applications which do not require the highest accuracy, the dichromate procedure for solid cuprous chloride is suggested. (For analysis of dilute solutions, a modification similar to that given for ceric ammonium sulfate may be used.) The *N* hydrochloric acid solution of ferric alum has the advantage of dissolving the cuprous chloride in only a few seconds.

Table IV. Effect of Acid Used in Ferric Ammonium Sulfate Solution

Acid Used as Ferric Alum Solvent[a]	Cuprous Chloride Found		
	$KMnO_4$	$K_2Cr_2O_7$	$Ce(SO_4)_2$
	%	%	%
N HCl	99.4	99.2[b]	99.6
6 *N* HCl	98.9	98.7	99.2
0.5 *M* H_2SO_4	99.3	99.2	99.6
3 *M* H_2SO_4	99.7	99.4	99.8[c]

[a] Solutions prepared in contact with air.
[b] Oxygen-free value = 99.5.
[c] Purity by electrodeposition = 99.8.

The stability and transparency of its solutions, its ease of standardization, and the high precision resulting from its use with the suggested indicator recommended potassium dichromate, instead of potassium permanganate, for general use as an oxidizing agent in cuprous chloride analysis. For the highest accuracy and dependability, the procedures using ceric ammonium sulfate should be employed, with oxygen-free solutions of ferric ammonium sulfate. Whatever the procedure, the chloride ion concentration should be kept low and an excess of ferric iron must be assured. It is recommended that the amount of ferric iron used be at least 1.5 times the theoretical. The precision then becomes that which is attained in the determination of ferrous iron using the same oxidizing agent. The accuracy to be expected of a particular procedure may be estimated from Table IV.

ACKNOWLEDGMENT

The authors would like to thank Gordon Sutherland for his help in part of the experimental work.

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Determination of Moisture in Sugar Solutions with the Karl Fischer Reagent

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Apparatus and procedure are described for determining moisture in sugar solutions with the Karl Fischer reagent. Data on representative analyses are given, with comparisons between refractometric determinations and Karl Fischer titrations.

THE Karl Fischer reagent has come into wide use for the quantitative determination of water in a variety of products. In a recent article, Johnson (*4*) gives an extensive bibliography.

This reagent, which is commercially available (*2*), consists of a solution of iodine and sulfur dioxide in a mixture of anhydrous methanol and pyridine. It is extremely sensitive to atmospheric moisture, and consequently titrations with it must be carried out in a completely closed system, especially where minute quantities of water are being determined. Furthermore, the necessity for stirring the reaction mixture during the titration introduces an extra difficulty into the design of a suitable apparatus.

The equipment described below is rugged and overcomes all the manipulative difficulties.

APPARATUS

Two 50-ml. automatic burets (Scientific Glass Apparatus Co. J-740) are so modified that the lower ends of the barrels of the Fresenius stopcocks are enlarged and formed into the outer parts of semiball 18/9 joints (Figure 1). The titration vessel (Figure 2) is a flat-bottomed extraction flask of 250-ml. capacity provided with a mouth, which is a male semiball 65/40 joint, and a ⅁14/35 side tube through which go the mutually insulated platinum electrodes sealed into a tube with a male ⅁14/35 joint and connected to the brush posts of the titrimeter. The flask is covered with a bell-shaped dome, the lower part of which is a female semiball 65/40 joint. From the top of the dome project two male semiball 18/9 joints set at sufficiently differing heights to permit free rotation of the buret stopcocks. In addition, the dome is provided with a check valve (Scientific Glass Apparatus Co. J-4880 B) to permit venting of the apparatus whenever liquid is being admitted into the flask from the burets. The male 18/9 joints fit into the burets which are provided with stopcock plugs with tips 16.5 cm. long.

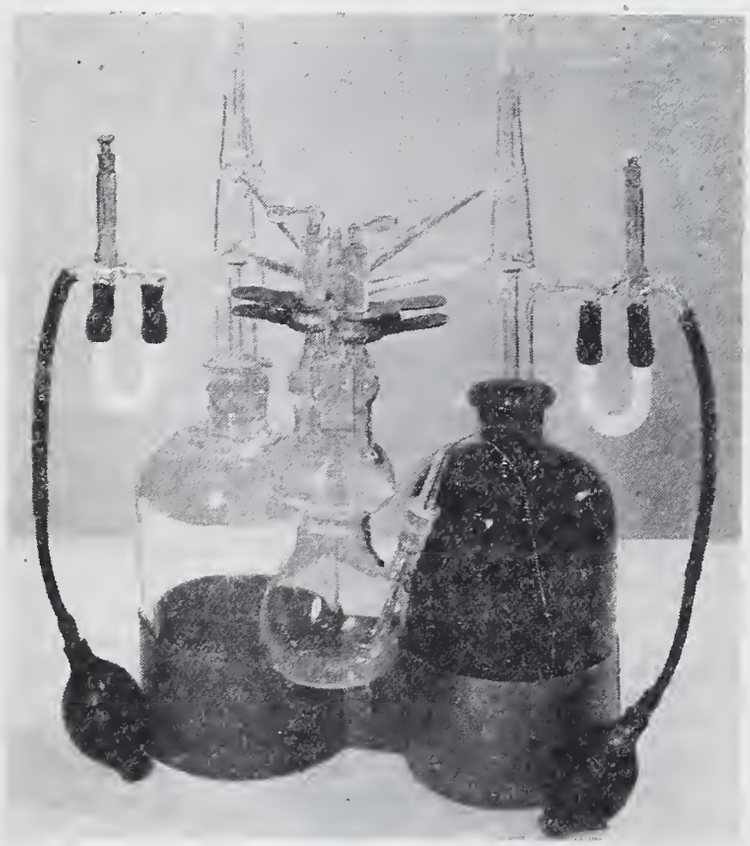

Figure 1. Glass Assembly of Titration Apparatus

Stirring is accomplished by means of a piece of iron bolt 2.81 cm. (1.125 inches) long and 0.47 cm. ($^3/_{16}$ inch) in diameter, fitting snugly and sealed into a piece of Pyrex tubing, the center of which has been blown out into a small bulb. An Alnico horseshoe magnet (Fisher Scientific Co. 12-012), mounted on a motor provided with a rheostat, is placed under the flask.

Table I. Standardizations of the Solutions

Standard Methanol *vs.* Karl Fisher Reagent

K.F. Reagent *Ml.*	Standard Methanol *Ml.*	1 Ml. of Alcohol ≏ *a* Ml. of K.F.
13.45	40.70	0.3305
13.90	42.20	0.3294
13.60	41.05	0.3313
		Av. 0.3304

Titration of Known Weights of Water

Water *Gram*	K.F. Reagent *Ml.*	Standard Methanol Back-Titration	Ml. of K.F. ≏ to Standard Methanol	Net K.F. *Ml.*	1 Ml. of K.F. ≏ Gram of Water
0.1020	26.90	3.50	1.16	25.74	0.003963
0.1145	29.75	2.50	0.83	28.92	0.003959
0.1241	31.95	2.30	0.76	31.19	0.003979
					Av. 0.003967

Water Equivalent of Standard Methanol

Standard Methanol *Ml.*	≏ K.F. *Ml.*		*Gram water/ml. methanol*
40.70	≏ 13.45	× 0.003967 =	0.001311
42.20	≏ 13.90	× 0.003967 =	0.001307
41.05	≏ 13.60	× 0.003967 =	0.001314
			Av. 0.001311

PROCEDURE

In operation, the apparatus is assembled, and a measured amount of Karl Fischer reagent is run into the flask. The stirrer is started, and standard methanol is rapidly added from the other buret until the solution, which originally had the appearance of tincture of iodine, turns to a light, reddish brown. The methanol is then added slowly, and finally dropwise until the end point is reached. With the Fisher Scientific Co.'s titrimeter employing a polarized platinum-platinum electrode pair, this is indicated by the closing of the "magic eye" (*2*). Fresh Karl Fischer reagent turns to a chromate yellow, but an old solution remains brownish at the end point, making it more difficult to gage visually the final stage of the titration. For maximum sensitivity, it is important that the platinum wires be perfectly clean, and it is advisable to bathe them for an hour or two in strong, hot dichromate–sulfuric acid cleaning solution.

It was found that the absolute methanol sold by the Carbide and Carbon Chemicals Corporation contains approximately 0.0013 gram of water per milliliter, which makes it excellently suitable to use for back-titrations, because it is roughly only one fourth as strong as the average Karl Fischer reagent. Three or four determinations are made in this manner, and the average value is expressed as:

1 ml. of standard methanol ≏ *a* ml. of Karl Fischer reagent

Next, from a small weight pipet, 2 coarse drops of water, weighing about 0.1200 gram, are removed, added directly to 25 ml. of Karl Fischer reagent in the flask, and immediately back-titrated with the standard methanol. The burets are read to the nearest 0.01 ml. The weight of the water is noted accurately to 0.1 mg. A 15-ml. Erlenmeyer flask with ⅁19/10 outside grind, provided with a cover through which is sealed a medicine dropper, and fitted with a small rubber bulb, is recommended for weighing out the water, as well as samples of sirup. A minimum of three such samples of water are weighed out, and back-titrated with the standard methanol. From this information, coupled with the alcohol–Karl Fischer reagent equivalence previously determined, it is possible to calculate: (1) the number of milligrams of water

equal to 1 ml. of Karl Fischer reagent, and (2) the number of milligrams of water in each milliliter of alcohol used in the back-titrations.

The sample of sirup is treated exactly like the water used in the standardization of the solutions. Three to five coarse drops, depending on the water content, are carefully weighed out and added to 20 to 25 ml. of the Karl Fischer reagent, and immediately back-titrated with the standard methanol. A sample series of determinations is shown in Tables I and II.

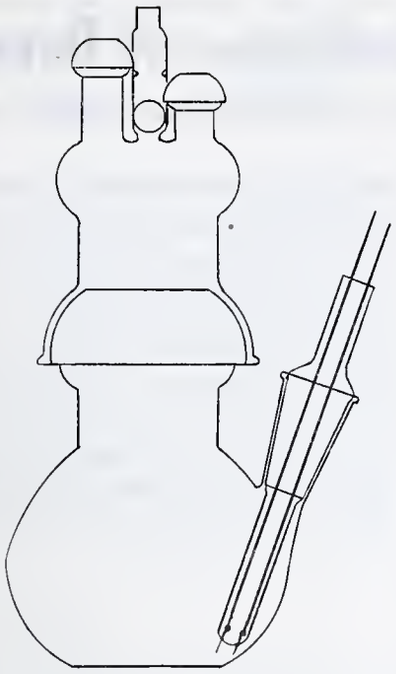

Figure 2. Titration Flask and Dome, Showing Arrangement of Ground-Glass Joints

An examination of the data shows that the milligrams of water found check with the calculated values obtained by means of a Bausch & Lomb precision sugar refractometer and tables corresponding to each particular sugar, within the limits found by other workers. Reporting of water content in terms of milligrams found and calculated introduces a fallacious, high precision. Differences of 2 mg. are commonly reported in the literature, but, expressed in per cent, agreement between known and found amounts of water is not ideal, as is revealed by comparing the per cent water found, column 7, with the observed values, column 8. The per cent discrepancy is obviously correlated with the total amount of water that is present.

While close agreement between refractometric determinations and Karl Fischer titrations of chemically pure sugar solutions can be expected, this is not so in those cases where soluble impurities are present, or the material is a mixture of sugars of unknown proportions. For routine work, certainly, the Karl Fischer titrations give satisfactory results under those conditions where the refractometer is not applicable. It, however, does not have the precision and accuracy of an acid-base titration.

The recent work of Aepli and McCarter (*1*) indicates that the change in the water equivalence of the Karl Fischer reagent with time is a highly important matter. A fresh solution rapidly falls off in strength, and these authors show that a solution 22 hours after preparation had a value of 1 ml. = 5.3 mg. of water, whereas 18 hours later it had fallen to 4.6. This represents a 14% change, so that even during a working day there is a likelihood of a significant change. There is no way of telling just how temperamental a given solution may be, and hence it is best to make a determination of an unknown sample shortly before or after the standardizations of the solutions. Frediani (*3*) advises that the analyst should age his solution for a week before using it, at which time, "if the solution is a good one, a relatively stable reagent will be available".

For critical work, it might be necessary to standardize the Karl Fischer reagent before and after each determination of a given sample, and to use the average values. Fortunately, the standard methanol, if it is properly protected, does not change appreciably and can be used as a secondary standard. However, its water content should be redetermined every day.

The Karl Fischer method, when used with sugar solutions containing 20 or more % water, has certain limitations and inherent sources of error which should be borne in mind. The solution is expensive, and 1 liter of it is used up by approximately 5 ml. of water. The reagent has a rather high viscosity, and buret drainage therefore becomes a serious factor. To overcome these difficulties, the reagent might better be diluted with a mixture of absolute methanol–pyridine (3 to 1), so that its water equivalence is between 2 and 3 mg. per ml. High-grade pyridine must be used to assure stability. It is obvious that a buret reading must be at least 15 to 20 ml. before calibration errors in an uncalibrated buret, and the drainage and reading errors become sufficiently small relative to the total volume used, not to introduce too large percentage errors. Furthermore, the reagent decomposes on standing and produces an oily film in the buret. It is therefore convenient to have two Karl Fischer reagent burets available, so that a clean and dry one is ready at the beginning of each day.

Samples of sugar solutions containing large amounts of water force one of three choices: (1) a small sample weighing 60 to 70 mg., for the sake of economy, (2) a sample two or three times that amount in order to enable more precise weighings, or (3) a large sample, diluting with absolutely anhydrous methanol and taking an aliquot. The first choice is not desirable in ordinary work because it would require calibrated weights, or the use of a microbalance. Choice 3 introduces the problem of preparing, storing, and handling an anhydrous hygroscopic liquid.

The work is being continued with commercial sirups and molasses in an endeavor to determine the relationship between vacuum drying of samples and the Karl Fischer titration.

Table II. Analyses of Sugar Solutions[a]

1 Sugar	2 Weight	3 H_2O Found	4 H_2O Calcd.	5 Difference	6 H_2O Found	7 Av.	8 H_2O, Refractometer
	Gram	*Mg.*	*Mg.*	*Mg.*	%	%	%
Dextrose	0.1152	68.1	69.2	−1.1	59.13		
	0.1119	65.9	67.3	−1.4	58.92	59.03	60.11
	0.0901	53.8	53.7	+0.1	59.72		
	0.1441	86.1	85.8	+0.3	59.74	59.73	59.57
Sucrose	0.1941	67.0	67.1	−0.1	34.53		
	0.2036	71.1	70.4	+0.7	34.92		
	0.1845	63.0	63.8	−0.8	34.13	34.73	34.59
	0.2172	74.9	75.5	−0.6	34.48		
	0.3020	107.2	105.0	+2.2	35.50		
	0.3546	121.8	123.3	−1.5	34.35	34.78	34.77
	0.4438	109.0	107.4	+1.6	24.56		
	0.3123	77.0	75.6	+1.4	24.65		
	0.3577	88.4	86.6	+1.8	24.73	24.65	24.21
Levulose	0.3931	76.5	78.3	−1.8	19.44		
	0.3715	73.6	74.0	−0.4	19.82		
	0.3847	75.0	76.6	−1.6	19.51	19.59	19.92
Commercial invert sirup	0.2678	77.0	76.1	+0.9	29.03		
	0.2419	70.1	68.2	+1.9	28.99	29.01	28.42

[a] Representative determinations made on five different days, involving completely new standardizations each day. Karl Fischer reagents of fourfold variations in strength are included. However, there is no apparent correlation between concentration of reagent and precision.

ACKNOWLEDGMENTS

The writers wish to express their thanks to William Geyer of the Scientific Glass Apparatus Co. for his cooperation in the manufacture of the apparatus, to Harold A. Frediani of Eimer and Amend for his generous advice and constructive criticism, and to Frederick Bates of the National Bureau of Standards for kindly supplying a sample of pure levulose.

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Apparatus for Flash-Distillation of Butadiene

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An apparatus for the flash-distillation of butadiene is described and a diagram shown. Its efficiency has been determined with the aid of the Dorell weathering-test apparatus. Substantially complete removal of butadiene dimer is accomplished without appreciable loss of C_5 hydrocarbons.

IN THE analysis of butadiene, the determination of C_5 hydrocarbons by a Dorell (Dow-Cottrell) weathering tester (*2*) gives high percentages if butadiene dimer is present. Preliminary to the determination it is usually necessary to carry out a flash-distillation of the sample to remove dimer (vinyl cyclohexene) and high-boiling material. Previous methods of doing this have been defective in failing to give a complete removal of dimer without loss of the C_5's which should remain in the volatile fractions. To remedy this difficulty, the apparatus described below has been developed.

APPARATUS

The apparatus (Figure 1) consists of the following parts: *F* is an ampoule forming the kettle of the apparatus. A neck, *G* (15 cm. long), connects the kettle with cup *D*, having an inner cup, *H*. From *H* a closed tube, *T*, extends through the neck and just into the kettle. A cork, *E*, is inserted in the closed tube to keep materials placed in the cup from entering the tube. *C* is a side arm through which the apparatus is filled and emptied. The apparatus is made of Pyrex to lessen the danger of breakage by sudden changes of temperature.

OPERATION

The ampoule is evacuated through *C*, closed off, and the apparatus cooled in a dry ice bath (dry ice in a mixture of chloroform and carbon tetrachloride, 50–50 by weight, *1*). The sample to be flash-distilled is introduced into the cooled, evacuated ampoule. A trap is connected to *C*, evacuated, and closed off at *A*. Cup *H* is filled with a dry ice bath and an excess of dry ice is maintained in the cup throughout the distillation. The trap is opened into the ampoule, the bath is removed from around it, and the trap is placed therein. Caution should be used to keep out all air or noncondensable gases at dry ice bath temperature. Material that does not condense will prevent the distillation of the butadiene, since the distillation is carried out in a closed system. The dry ice bath in the cup maintains a reflux of butadiene separating the dimer and high-boiling material from the butadiene and C_5's. If the cork is not in the tube, a heavier reflux is caused, requiring a longer time for distillation; with the cork in place about an hour is required. When the ampoule has come to room temperature, the trap is closed at *B* and the sample is ready

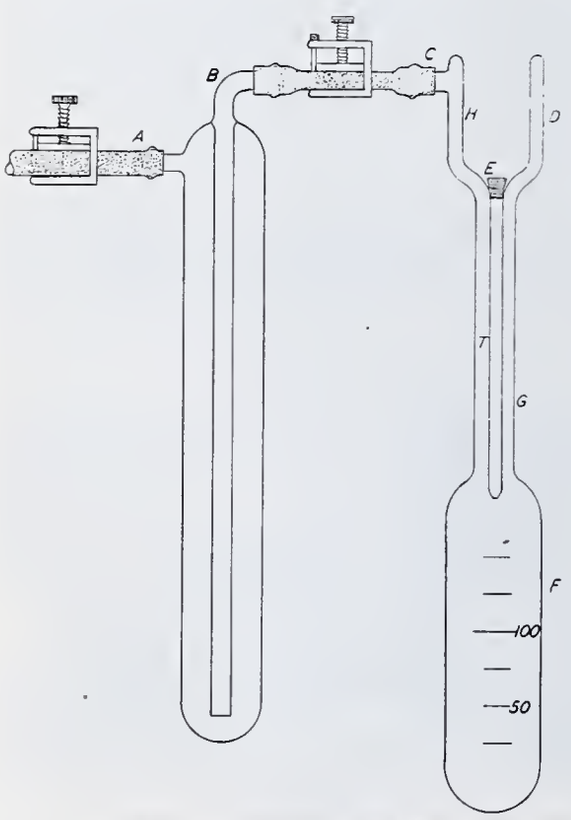

Figure 1. Ampoule and Trap

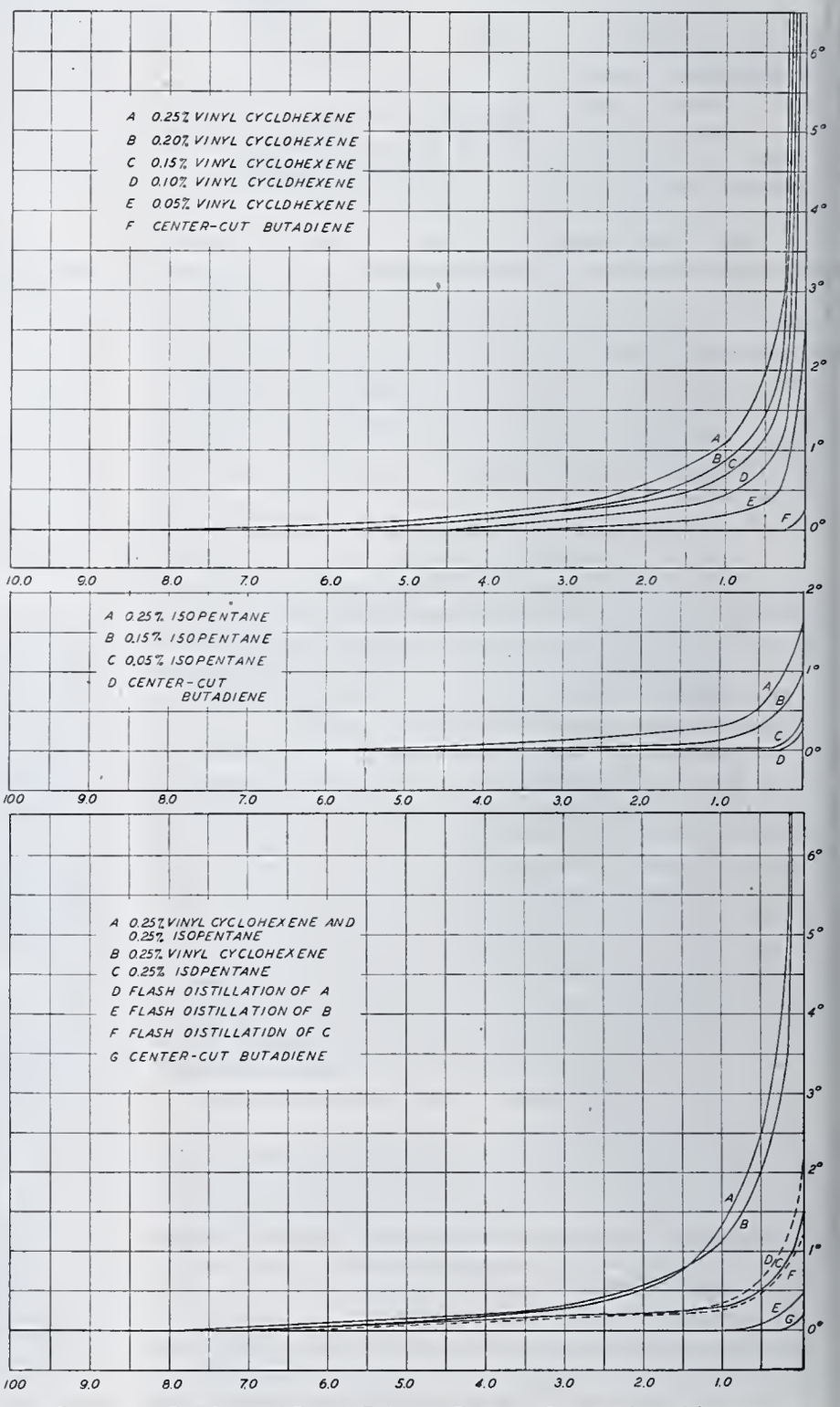

Figure 2. Readings of Dorell Tube *vs.* Degrees Centigrade of Variation

Table I. Temperature Readings

Residual Volume *Ml.*	Temperature ° *C.*	Temperature Difference ° *C.*
10.0	−4.5	...
9.0	−4.7	...
8.0	−4.7	0.0
7.0	−4.7	0.0
6.0	−4.7	0.0
5.0	−4.7	0.0
4.0	−4.7	0.0
3.0	−4.7	0.0
2.5	−4.7	0.0
2.0	−4.6	0.1
1.5	−4.6	0.1
1.0	−4.6	0.1
0.8	−4.6	0.1
0.6	−4.6	0.1
0.4	−4.6	0.1
0.2	−4.5	0.2
0.1	−4.5	0.2
Pump stops	−4.4	0.3
Dry point	−4.3	0.4

for C_5 determination or any other determination requiring a flash-distilled sample.

DISCUSSION

In order to test the efficiency of the apparatus, butadiene samples have been submitted to flash-distillation and then analyzed in the Dorell weathering-test apparatus. For the determination of C_5 hydrocarbons, 40 ml. of distilled sample are placed in the boiler of the Dorell and 30 ml. are allowed to "weather away" before the heat is turned on and temperature readings are taken. These readings are taken at 10.0, 9.0, 8.0, 7.0, 6.0, 5.0, 4.0, 3.0, 2.5, 2.0, 1.5, 1.0, 0.8, 0.6, 0.4, 0.2, and 0.1 ml., and when the pump stops and the boiler becomes dry. A typical record is given in Table I.

The differences starting at 8.0 ml. between the initial and succeeding temperatures observed are plotted. This curve is compared with curves obtained by plotting the temperature differences of butadiene using known amounts of isopentane run in the same way. Temperature readings at 10.0 and 9.0 ml. are disregarded because the thermometer is still in the liquid phase only and will not give a true boiling point.

Figure 2 (upper) shows the curves produced by center-cut butadiene with varying amounts of butadiene dimer added. Curve *F* is made by plotting temperature differences of center-cut butadiene. It gives almost a straight line, as would be expected. There are significant differences between the curves obtained with varying amounts of dimer.

Figure 2 (center) shows butadiene with different amounts of isopentane. In Figure 2 (lower) are plotted curves obtained with the following mixtures:

A. Butadiene with 0.25% dimer and 0.25% isopentane, not flash-distilled.
B. Butadiene with 0.25% dimer, not flash-distilled.
C. Butadiene with 0.25% isopentane, not flash-distilled.
D. Curve after flash-distillation of *A*.
E. Curve after flash-distillation of *B*.
F. Curve after flash-distillation of *C*.
G. Curve of center-cut butadiene.

Curve *D* shows the almost complete removal of dimer and the retention of C_5's with the butadiene. Curve *E* is representative of the removal of dimer with no C_5's present. The amount of dimer left after flash-distillation with this apparatus expressed as C_5's would be approximately 0.05% which is very small. Curve *F* shows the effect of the flash-distillation on C_5's.

Although the flash-distillation apparatus described was designed specifically for removing dimer and other high-boiling materials from butadiene containing C_5's, it is believed to offer possibilities for other separations. Reflux ratios might be changed by the use of different cooling media or by allowing tube *T* to be filled with coolant.

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Simple Hydrostatic Gravitometer

For Rapid Determination of the Specific Gravity of Liquids

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An apparatus is described for the rapid, precise determination of the specific gravity of selected liquids. Its use is based upon the principle that the change in height of two columns of liquids exposed to the same change in vacuum (or pressure) is inversely proportional to the specific gravity. When one liquid is of known specific gravity, the specific gravity of the other may be calculated. The apparatus is simple, easily constructed, and inexpensive, and is particularly suitable for determining the specific gravity of a large number of similar samples in control testing. The standard deviation of a single value from the value obtained by pycnometer is $\pm 1.4 \times 10^{-4}$ specific gravity unit. The speed with which the determination may be made makes it practical to improve this agreement by calculating the average of a number of determinations on a single sample.

IT IS a well-known principle of hydrostatics that the heights to which two columns of liquids of different specific gravities will rise when exposed to the same vacuum are inversely proportional to their specific gravities. This principle has been applied to the measurement of specific gravities.

Ciochina (*1*) described an apparatus for this purpose which consisted of two U-tubes placed side by side, with a millimeter scale between two of the arms—one from each U-tube—and with those arms joined. By means of a system of stopcocks water is placed in one U-tube and the sample in the other. The levels are read on the millimeter scale, pressure or vacuum is applied through the joined arms, and the levels are read again. From the change in levels and the known specific gravity of the standard liquid (water), the specific gravity of the liquid to be measured may be calculated by a simple inverse proportion. Accuracy to within a few units in the fourth decimal place was reported, but the authors were not able to confirm this. Some of the difficulty may have been caused by the stopcocks which are part of this apparatus. Another disadvantage of this apparatus is that it is necessary (according to Ciochina) to use a vernier in order to get the precision reported.

Another apparatus based on the same hydrostatic principle has been proposed by Davidson and co-workers (*2*, *3*). The liquid under examination is placed in a Z-tube, so that its height is fixed, and the standard liquid is placed in an L-tube manometer. By proper graduation of a scale fixed to the L-tube, the specific gravity of the liquid in the Z-tube may be read. A precision of from 0.1 to 0.2% is claimed (in other words the specific gravities are readable only to the third decimal place). This apparatus is constructed for use with small volumes of liquid.

Frivold (*5*) described an apparatus, which was claimed to be precise to $\pm 10^{-7}$, but it is too complicated for rapid routine use.

This paper describes an apparatus which is based on the same hydrostatic principle as those just mentioned, but has certain ad-

vantages over them. The apparatus may be constructed in a short time from simple equipment found in most laboratories. There are no essential stopcocks which can get out of order, and it gives specific gravities which are comparable in accuracy to those obtained by a pycnometer under ordinary conditions of control work. The apparatus is most suitable for rapid control work where specific gravities must be determined for a large number of samples of selected liquids of similar composition. Accurate values should be obtainable by workers of insufficient skill to be trusted with pycnometers, Westphal balances, or high precision hydrometers. Although no work was done with samples of limited volume, an extension of the method to such samples is conceivable.

Figure 1

APPARATUS

The apparatus (Figure 1) consists of two 150-cm. (5-foot) lengths of glass tubing of as nearly equal inside diameter as can be found (both 3- and 6-mm. inside diameter tubings have been used), fixed on either side of a meter rule, joined at the top to a Y-tube by tightly fitting rubber connections, and bent, as shown, at the bottom. The Y-tube is attached to a source of vacuum which can be regulated or released by means of a screw clamp and a stopcock attached to a rubber tube. Both the upper and the lower 10 cm. of the glass tubing and the meter rule must be left unobstructed in order to enable readings to be made. The lower ends of the apparatus dip into 50- or 100-ml. beakers chosen to be as nearly alike as possible in diameter. One beaker is for the sample, the other for the standard liquid. The apparatus must be kept scrupulously clean and must be placed where there are no drafts or sudden changes in temperature.

PROCEDURE

One side of the apparatus is rinsed with the liquid to be examined and the other side with the standard liquid. The standard liquid is preferably of similar composition to the sample; in fact, it may under some circumstances very well be part of an actual batch of the type of liquid to be controlled whose specific gravity has been carefully determined by pycnometer or Westphal balance. The beakers are filled to about two thirds of their capacity with their respective liquids, keeping the final level well below the flare of the beaker. The tubes are immersed to within about 1 cm. of the bottoms of the beakers. By a gradual application of vacuum the liquid columns are raised nearly to the top of the scale, the stopcock is closed, and readings are made of the positions of the two menisci (estimating to the smallest possible fraction of a millimeter). The two readings should be made as close together in time as possible, so that there will be no changes in the positions of the menisci while the readings are being made. Subsequent changes are of no effect. The vacuum is then released slowly, to allow for drainage as the columns drop, and when the menisci are at about 5 cm., the stopcock is again closed and readings are repeated.

For extremely volatile liquids with which a vacuum might be undesirable, pressure could be used, by having the sample and standard in closed flasks attached to a common source of pressure. The changes in technique that might be required should be obvious. This modification might also be desirable for very hygroscopic liquids, in which case it would be possible to protect the liquids against access of atmospheric moisture by drying tubes.

CALCULATIONS

General Method. Let *hsa* and *hst* equal the readings for sample liquid and standard liquid, respectively, at the top of the columns, in cm.

h'sa and *h'st* = the corresponding readings at the bottom of the columns, in cm.

Sst = the specific gravity of the standard at t/t'

Ssa uncorrected = the specific gravity of the sample at t/t'

$hst - h'st = A$ and $hsa - h'sa = B$

Then
$$Ssa \text{ uncorrected} = Sst\ A/B \qquad (1)$$

To *Ssa* uncorrected there must be added or subtracted a small "asymmetry correction" for the apparatus, which is discussed below.

Approximate Method. For many purposes a less exact but more rapid method of calculation may be used. By this method

$$Ssa \text{ uncorrected} \cong Sst + 0.01\ (A - B) \qquad (2)$$

This method of calculation, although merely an approximation, will give values which are fairly close to the true value, provided the term $(A - B)$ is small and provided 100 *Sst* is not much different from *B*.

The correct increment to be added to *Sst* to obtain *Ssa* may be calculated from Equation 1 in terms of $A - B$ and B (rather than by calculating *Ssa* from the ratio A/B) by substituting for A/B the equivalent term $1 + (A - B/B)$. (The latter is obtained from A/B by adding $B - B$ to the numerator.) Equation 1 then becomes

$$Ssa \text{ uncorrected} = Sst\ [1 + A - B/B)\ ] = Sst + Sst\ (A - B/B)$$

or

$$Ssa \text{ uncorrected} - Sst = Sst\ (A - B/B) \qquad (3)$$

Table I gives a comparison of the values of the increments calculated for benzene (*Sst* = 0.88) by means of both Equations 3 and 2. From this it may be seen that under appropriate conditions even the approximate method of calculation leads to only small errors.

Nomographs. Nomographs may be constructed to avoid most of the calculations, but in order to get readings to the fourth decimal place, the nomographs would have to be restricted to narrow specific gravity ranges.

Table I. Comparison of Increments Calculated by Exact and Approximate Methods

Assumed Value A−B	Assumed Value B	Increment to Be Added to *Sst*[a]: Exact calculation by Equation 3	Increment to Be Added to *Sst*[a]: Approximate calculation by Equation 2
Cm.	*Cm.*		
0.50	88	0.0050	0.0050
0.50	92	0.0048	0.0050
0.50	84	0.0052	0.0050
0.30	88	0.0030	0.0030
0.30	92	0.0029	0.0030
0.30	84	0.0031	0.0030
0.10	88	0.0010	0.0010
0.10	92	0.0010	0.0010
0.10	84	0.0010	0.0010

[a] When $A - B$ is positive, the exact or the approximate increment will be positive; when $A - B$ is negative, they will be negative.

Table II. Specific Gravity Determinations

Sample No.	Specific Gravity, 15.5° C./15.5° C.: By pycnometer	Specific Gravity, 15.5° C./15.5° C.: By hydrostatic method	Hydrostatic Average Minus Pycnometer Value × 10⁴
Benzene			
1	..	0.8835, 0.8835, 0.8835, 0.8834, Av. 0.8835	...
2	..	0.8822, 0.8823, 0.8823, 0.8822, Av. 0.8823	...
3	..	0.8823, 0.8822, 0.8823, 0.8824, Av. 0.8823	...
4	..	0.8837, 0.8835, 0.8836, 0.8836, Av. 0.8836	...
5	..	0.8825, 0.8824, 0.8825, 0.8824, Av. 0.8825	...
6	..	0.8823, 0.8822, 0.8823, 0.8823, Av. 0.8823	...
Toluene			
1	0.8707	0.8708, 0.8709, 0.8708, 0.8710, Av. 0.8709	+2
2	0.8707	0.9708, 0.8708, 0.8708, 0.8710, Av. 0.8709	+2
3	0.8705	0.8706, 0.8705, 0.8706, 0.8705, Av. 0.8706	+1
4	0.8708	0.8708, 0.8708, 0.8709, 0.8710, Av. 0.8709	+1
5	0.8706	0.8706, 0.8707, 0.8709, 0.8708, Av. 0.8708	+2
6	0.8708	0.8708, 0.8708, 0.8708, 0.8708, Av. 0.8708	0
Xylene			
1	0.8678	0.8677, 0.8678, 0.8677, 0.8676, Av. 0.8677	−1
2	0.8677	0.8675, 0.8676, 0.8673, 0.8674, Av. 0.8675	−2
3	0.8687	0.8687, 0.8688, 0.8688, 0.8686, Av. 0.8687	0
4	0.8686	0.8683, 0.8684, 0.8686, 0.8685, Av. 0.8685	−1

Table III. Specific Gravity Determinations

Sample	Specific Gravity 25°/25° C. By pycnometer	By hydrostatic method	Hydrostatic Average Minus Pycnometer Average × 10^4
1. *m*- and *p*-cresols	1.0325, 1.0324 Av. 1.0325	1.0324, 1.0326, 1.0326 1.0325	0
2. *m*- and *p*-cresols and xylenol intermediate	1.0285, 1.0285 Av. 1.0285	1.0285, 1.0285, 1.0286 1.0285	0
3. *o*-, *m*-, and *p*-cresols intermediate	1.0355, 1.0355 Av. 1.0355	1.0354, 1.0354, 1.0356 1.0355	0
4. Phenol, *o*-cresol intermediate	1.0626, 1.0625 Av. 1.0626	1.0627, 1.0627, 1.0627, 1.0626 1.0627	+1
5. *o*-Cresol	1.0431, 1.0430 Av. 1.0431	1.0431, 1.0431, 1.0431, 1.0430 1.0431	0
6. *o*-, *m*-, and *p*-cresols intermediate	1.0358, 1.0357 Av. 1.0358	1.0356, 1.0358, 1.0360, 1.0360 1.0359	+1
7. *m*- and *p*-cresols	1.0326, 1.0325 Av. 1.0326	1.0323, 1.0325, 1.0325, 1.0325 1.0326 1.0325	−1
8. Phenol, *o*-cresol intermediate	1.0605, 1.0605 Av. 1.0605	1.0603, 1.0603, 1.0603, 1.0603 1.0603	−2

CALIBRATION OF APPARATUS AND ASYMMETRY CORRECTION

Differences that may exist in the bores of the tubes and beakers for the sample and the standard will introduce a systematic error which should be determined for the apparatus under the same conditions as will actually apply in use, and with the same type of sample. The apparatus is calibrated by placing the same liquid on both sides and determining the value A/B, considering the liquid on that side of the apparatus corresponding to A as the standard and that on the other side as the sample. The "asymmetry" correction to be applied to *Ssa* uncorrected is then *Ssa* uncorrected (1.0000 − A/B). If, in actual determinations, the sample and the standard are measured on the same side of the apparatus as corresponds to the side for B and A, respectively, then the sign of the correction as calculated will be valid. The correction should be reproducible and should affect only the fourth decimal place.

RESULTS

Table II gives the results of specific gravity determinations of benzene, toluene, and xylene by pycnometer and by the hydrostatic method. In each case a sample of the same substance was used as a standard—i.e., benzene, toluene, and xylene were used as standards for benzene, toluene, and xylene, respectively. If any one of these was used as a standard for the other, errors in the third decimal place were produced.

Table III gives the results of specific gravity determinations of a number of cuts obtained in the distillation of tar acids, using No. 5 as a standard for Nos. 1 to 4 and No. 2 as a standard for Nos. 5 to 8. These results show that it is possible under appropriate conditions to use a standard liquid which is not necessarily the same as the sample, as contrasted with the measurements on benzene, toluene, and xylene. Attempts to use liquids and mixtures of somewhat similar specific gravity to the samples but otherwise dissimilar, such as mineral oil and water, led to errors up to 2 or more units in the third decimal place. Nitrobenzene yielded better values. Similar discrepancies were noted when benzene was used as a standard for toluene and xylene. Such properties as the temperature coefficient of expansion, the viscosity, and the surface tension might be important. The last two might affect drainage. If the first were different in the sample and in the standard, the assumption would not be justified that the specific gravity of the sample could be determined at any temperatures and the answer expressed at any other temperatures merely by using the proper value of the specific gravity of the standard at the desired temperatures.

It was found possible to use the gravitometer with a viscous liquid such as glycerol with apparently similar precision to that obtainable with, for example, water. However, no statement can be made regarding the applicability of the apparatus to viscous liquids in general; each liquid under consideration should be tested separately to see if its specific gravity can be determined with the requisite accuracy.

In a series of distillation cuts such as might be obtained, for example, in fractionating a mixture of methanol, ethanol, and considerable water, no one of these substances will serve satisfactorily as a standard for all the cuts. However, a single standard may be used—for example, methanol—in testing all the cuts, if a correction curve is constructed relating the correction to the specific gravity found.

ACCURACY AND PRECISION

The standard deviation from the pycnometer value of a single value obtained by the gravitometer is $\pm 1.4 \times 10^{-4}$ specific gravity unit. This figure represents the range about the pycnometer value within which about 68% of the determined gravitometer values will lie, and, of course, by taking the average of several determinations, which can be done rapidly, an even better value may be obtained. The deviation $\pm 1.4 \times 10^{-4}$ was calculated as described in the following paragraph.

Figure 2 is a frequency distribution diagram for the magnitude of the deviation between a single value by the gravitometer and the best available value by the pycnometer. By fitting a normal distribution to the variates as given in Figure 2 (*4*) a standard deviation of $\pm 1.46 \times 10^{-4}$ was calculated. It was shown by the X^2 test that the data of Figure 2 fit satisfactorily a normal distribution with a standard deviation of $\pm 1.46 \times 10^{-4}$. Since the standard deviation includes the errors of both the pycnometer and the gravitometer, and since a standard deviation for a single value by the pycnometer could be calculated as $\pm 0.44 \times 10^{-4}$ (from the data of Table III), a standard deviation of $\pm 1.4 \times 10^{-4}$ was calculated from these data (by the square root of the differences of their squares) as the standard deviation from the pycnometer value of a single gravitometer value. By applying Student's t test it was found that there was no reason to suspect a bias or constant error between the pycnometer values and the gravitometer values.

As a measure of precision, a standard deviation of a single value by the gravitometer from the average value was calculated (from

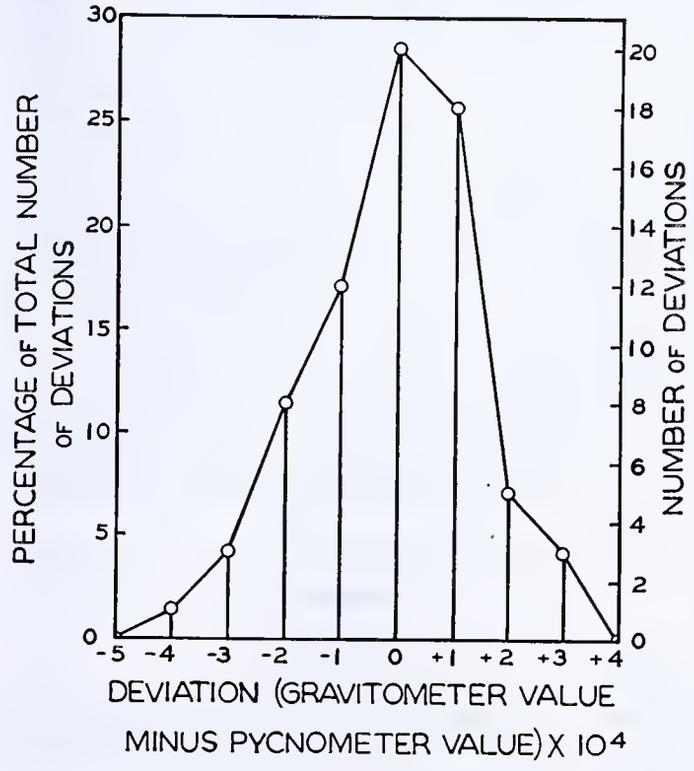

Figure 2. Frequency Distribution Diagram of Deviations of Pycnometer Values from Gravitometer Values

the data of Tables II and III) as $\pm 0.9 \times 10^{-4}$. The fact that this deviation is smaller than the above-mentioned deviation of $\pm 1.4 \times 10^{-4}$ from the pycnometer value, indicates that successive replicates agree better than replicates carried out over a longer period. Errors may be caused if both sides of the apparatus are exposed to different temperatures as by the action of drafts of air, and the poorer precision may perhaps be explained by the introduction of such variables over a period of time.

ACKNOWLEDGMENT. Grateful acknowledgment is made to E. I. Stearns for his assistance in the preparation of the discussion of accuracy and precision.

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Microdetermination of the Saponification Number of Fats and Oils

Decigram, Centigram, and Milligram Procedures

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THE literature contains no satisfactory method for the microdetermination of the saponification number of fats and oils. A semimicromethod was devised by Chargaff (*2*) but was applied to only three oils. It has been pointed out that the double-indicator method (*3*) is more easily adaptable to microprocedures than the standard method (*1*).

Methods are described for determination of the saponification number of fats and oils with samples of about 500, 50, and 5 mg.

APPARATUS

For the decigram procedure, a 10-ml. calibrated buret with 0.05-ml. graduations was used. The tip was constricted so that drops of about 0.01 ml. were delivered.

The buret for the centigram procedure is illustrated in Figure 1. A ground-glass rod, *A*, 5.2 mm. in diameter, fits snugly inside a ground-glass tube, *B*. The upper end of *B* is sealed to capillary, *C*, which is bent twice at right angles and constricted at the tip as indicated. A 25-mm. micrometer, *D*, the head of which has been removed, serves to measure accurately the position of the piston *A*. Above the piston is a layer of mercury, *E*, which makes a leakproof seal. Above the mercury is the 0.5 *N* hydrochloric acid. Castalloy clamps *F* hold the micrometer and glass tube rigidly in fixed positions. The titration vessel, *G*, is simply a 13 × 100 mm. Pyrex test tube. A movable arm, *H*, taken from a Rehberg buret made by Microchemical Service supports the titration vessel. A finely drawn capillary, *I*, admits a stream of air, free from carbon dioxide, into the titration vessel.

The assembly for the titration in the milligram procedure was essentially the same except that a smaller test tube (10 × 75 mm.) and ground-glass rod and tube (3.1 mm.) were used.

In order to refill these micrometer burets, the tip is wiped with Kleenex tissue and immersed in the 0.5 *N* hydrochloric acid. The micrometer spindle is turned down, and the piston is slowly pushed down with the fingers till it makes contact with the spindle.

A 4 × 3 × 2 mm. platinum boat was made from foil 0.06 mm. thick.

A fluorescent titration illuminator was used for all titrations.

REAGENTS

The alcoholic potassium hydroxide was prepared as previously described (*3*).

Alcoholic 0.001 *M* bromophenol blue was prepared by dissolving 65 mg. of the indicator in 1.0 ml. of 0.10 *N* sodium hydroxide and adding a mixture of 60 ml. of purified 95% alcohol and 40 ml. of benzene.

The alcohol-benzene mixture consisted of 60 ml. of reagent-grade benzene and 40 ml. of purified 95% alcohol.

Hydrochloric acid, approximately 0.5 *N*, was standardized with pure sodium tetraborate decahydrate and used in all titrations. The acid used for the centigram procedure was standardized by weighing accurately 400 to 600 mg. of a carefully prepared, approximately 0.5 weight-normal solution of borax into a 13 × 100 mm. Pyrex test tube, adding one drop of 0.001 *M* methyl red, and titrating to the first pink color. The results were expressed as milliequivalents of acid per millimeter.

The acid used for the milligram procedure was standardized analogously. About 10 mg. of pure borax were weighed on a microchemical balance and transferred to a 10 × 75 mm. Pyrex test tube. Four drops of water and one drop of 0.00008 *M* methyl red were added before the titration.

PROCEDURES

DECIGRAM PROCEDURE. Weigh accurately about 500 mg. of sample into a 50-ml. Pyrex Erlenmeyer flask. Add about 5 ml. of the alcoholic potassium hydroxide, and immediately connect a reflux condenser, the upper end of which is protected by an Ascarite tube. Boil gently for 30 minutes. Add 2 drops of 1% alcoholic phenolphthalein, and titrate with 0.5 *N* hydrochloric acid to the disappearance of the pink color. Add 3 drops (0.18 ml.) of aqueous 0.001 *M* bromophenol blue and 1 ml. of benzene. Continue the titration to a green color that does not turn blue on further agitation.

CENTIGRAM PROCEDURE. Weigh with an accuracy of 0.02 mg. about 50 mg. of sample into a tared Pyrex test tube, add about 21 drops (0.5 ml.) of alcoholic potassium hydroxide, and immediately attach an Ascarite tube. Support the tube vertically, so that the bottom rests on the flat surface of a micro drying block which is kept at 145° ± 5°. The test tube serves as both reaction vessel and reflux condenser. The saponification is complete in 30 minutes. Add 1 drop (0.03 ml.) of 0.2% alcoholic phenolphthalein. Insert the tip of the micrometer buret (previously wiped with Kleenex tissue) and the tip of the air-delivery tube, and support the tube in the titration apparatus (Figure 1). Adjust the air stream to a rate of about 80 bubbles per minute. Add the 0.5 *N* hydrochloric acid by carefully turning the micrometer spindle until the color just disappears. Read the micrometer at this point.

Without removing either capillary from the test tube, add 1 drop (0.06 ml.) of aqueous 0.001 *M* bromophenol blue and 7 drops (0.12 ml.) of benzene. Decrease the air stream to about 50 bubbles per minute, and continue the titration till the solution has a milky appearance; then add 7 more drops of benzene, and increase the air stream to about 100 bubbles per minute. Continue the titration to a green end point. The hydrochloric acid

used between the two end points is equivalent to the potassium hydroxide that was required for the saponification.

MILLIGRAM PROCEDURE. Weigh the platinum boat (4 × 3 × 2 mm.) on a microchemical balance, then place it on a clean surface. Dip the tip of a finely drawn glass rod into the sample, let most of the oil drain off the rod, and touch the center of the boat with the tip of the rod. Transfer the boat to the balance pan, and weigh it again. The sample should be between 3 and 8 mg. With platinum-tipped forceps, place the boat just inside the open end of a horizontally held 10 × 75 mm. Pyrex test tube. Then tilt the test tube so that the boat slides to the bottom of the tube. If any oil sticks to the side of the tube, the determination must be started anew.

Let 2 drops of the alcoholic potassium hydroxide fall directly from the siphon of the storage bottle into the bottom of this test tube. Immediately connect an Ascarite tube to the test tube. Then support it with a clamp and ring stand so that the bottom of the tube rests on the flat surface of a micro drying block heated to 130° ± 5°.

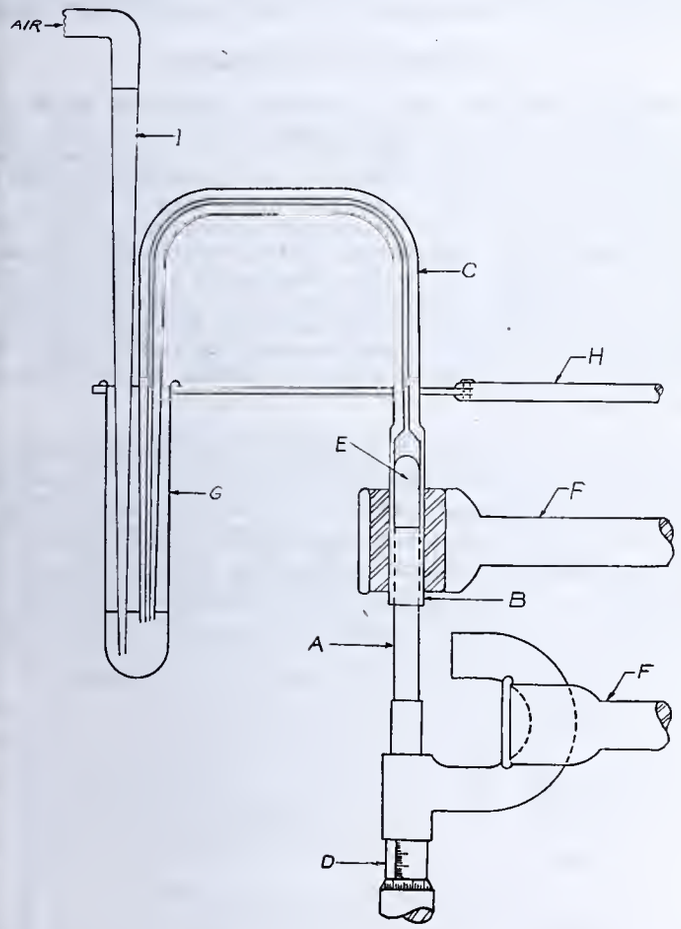

Figure 1. Micrometer Buret

After 30 minutes, remove the Ascarite tube and add 2 drops (0.06 ml.) of 0.02% alcoholic phenolphthalein. Immediately insert the tip (previously wiped with Kleenex tissue) of the micrometer buret and the tip of the air-delivery tube, and support the tube in the titration apparatus (Figure 1). Take care that the boat does not interfere with the delivery of acid from the buret. Adjust the air stream to about 60 bubbles per minute. Note the reading, a, of the micrometer before any hydrochloric acid is added to the titration vessel. Now titrate to the disappearance of the pink color of the phenolphthalein. Rinse the inside of the test tube with 0.10 to 0.20 ml. of the alcohol-benzene mixture by means of a measuring pipet. If the pink color reappears, add more hydrochloric acid till it disappears again. Note this reading, b, of the micrometer. Add 2 drops (0.06 ml.) of the alcoholic bromophenol blue. Continue the titration. Just before the end point is reached, rinse the inside of the test tube again with 0.10 to 0.20 ml. of the alcohol-benzene mixture. Note the micrometer reading, c, when the green end point is reached.

Contamination of the alcoholic potassium hydroxide by carbon dioxide of the air could not be avoided in the milligram procedure, although no difficulty was encountered in this respect in the other procedures. Therefore a blank correction must be applied in the milligram procedure. To run the blank, put 2 drops of the alcoholic potassium hydroxide into the Pyrex test tube and titrate it as described above, noting the initial micrometer reading, x, the reading at the phenolphthalein end point, y, and the reading at the end point of bromophenol blue, z. Then calculate the saponification number with the equation

$$S = \frac{[(b - c) - B(a - c)]56.11\ F}{W}$$

where S = saponification number

$$B = \frac{y - z}{x - y}$$

F = concentration of hydrochloric acid, milliequivalents per mm.

W = weight of sample, grams

RESULTS

The saponification numbers of eleven oils were determined by the standard method (*1*) and by each of the methods described in this paper. Each entry in Table I denotes a single determination except as indicated in the footnotes. No values were omitted except those obtained before the methods had been perfected.

DISCUSSION

Excellent results are obtained by the decigram and centigram methods, while the milligram method yields very satisfactory results. In order to study the precision of the methods, one oil was run several times by the same method. The mean deviations were 0.3, 0.1, and 0.5 unit by the decigram, centigram, and milligram methods, respectively.

Table I. Comparison of Results by Various Methods

Oil	Saponification No. Standard method	Saponification No. Decigram method	Difference	Saponification No. Centigram method	Difference	Saponification No. Milligram method	Difference
Castor oil	181.5	181.5	0.0	182.0	+0.5	182.4	+0.9
Cocoa butter	194.6	194.5	−0.1	195.0	+0.4	195.4	+0.8
Coconut oil	258.6	258.9	+0.3	258.5	−0.1	259.2	+0.6
Cod liver oil	188.0	188.6	+0.6	187.9	−0.1	188.2[a]	+0.2
Corn oil	191.6	191.6[b]	0.0	191.6[a]	0.0	192.1	+0.5
Lard oil	195.5	195.6	+0.1	195.7	+0.2	196.2	+0.7
Linseed oil	187.0	187.1	+0.1	187.4	+0.4	187.4	+0.4
Neat's-foot oil	186.7	186.7	0.0	186.4	−0.3	187.4	+0.7
Olive oil	191.5	191.8	+0.3	192.0	+0.5	190.7	−0.8
Rapeseed oil	174.3	174.0	−0.3	174.6	+0.3	175.2	+0.9
Tung oil	194.7	194.5	−0.2	194.4	−0.3	195.0	+0.3
Mean (signs disregarded)			±0.2		±0.3		±0.6

[a] Mean of 4 determinations.
[b] Mean of 10 determinations.

The decigram method is more convenient than the gram method chiefly because the smaller solution cools almost instantaneously after saponification and reduces the waiting period. The centigram procedure is probably a little more troublesome because of the special buret. The milligram procedure is still more troublesome and is to be chosen only when the quantity of sample is very limited.

None of these methods is applicable to acetylated oils (*3*).

ACKNOWLEDGMENT

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Colorimetric Micromethod for Determination of Antimony in Biological Materials

With Concomitant Determination of Bismuth

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A colorimetric method for the determination of antimony in biological materials is presented. The material is digested in sulfuric acid with the addition of nitric acid or of a mixture of nitric and perchloric acids. The diluted digest is treated with a solution containing potassium iodide and ascorbic acid; the color of the potassium iodoantimonite complex is determined in a photoelectric colorimeter. The standard error is about 2%, and the recoveries of added antimony are usually within ±2%. Quantities as small as 10 micrograms may be estimated, but the error here is somewhat larger. Bismuth gives the same color reaction as antimony. However, if it is present, it may be quantitatively determined on the same digest, and by subtraction the amount of antimony may be estimated.

PROGRESS in the experimental study of the organic compounds of antimony has thus far been limited by the lack of a simple and accurate method for the determination of the amounts of that element likely to be found in the body tissues, fluids, and excreta, particularly the urine (*3*). The method of Christiansen (*1*) is applicable to all types of biological material but requires the presence of rather large amounts of antimony. Hassan (*6*) has published a technically involved method which he applied to urine only. The rhodamine B method of Fredrick (*4*) is very specific, and accurate, but is also technically complicated; while it could undoubtedly be adapted to biological material, the author did not so apply it. Very recently Webster and Fairhall (*8*) have employed the same reagent for the determination of microquantities of stibine.

An excellent lead for a method has been provided by the work of Fauchon (*2*). He has shown that the addition to solutions of Sb^{III} of a reagent consisting of a solution 10% in both sulfuric acid and potassium iodide yields the yellow complex, $KSbI_4$, which can be determined colorimetrically. From these observations the present method has been developed.

In this work it has been deemed more practicable to add the sulfuric acid and the potassium iodide separately rather than as a single reagent, for two reasons: (1) the reagent described by Fauchon is very unstable, and (2) in the acid digests of biological material which it was proposed to use in this work, sulfuric acid would already be present. Its concentration in these digests could readily be adjusted to the proper point as determined experimentally, and it could therefore be omitted from the color reagent.

REAGENTS

Sulfuric acid, specific gravity 1.17, $\frac{20°}{4°}$ C., 160 ml. of concentrated acid diluted to 1 liter with distilled water (referred to below as 16 volumes % sulfuric acid).

Potassium iodide reagent A, 112 grams of C.P. potassium iodide and 20 grams of crystalline ascorbic acid dissolved in distilled water to make 1 liter. This reagent is made up only as needed and is kept in a brown bottle, but is stable for about one month. It must be discarded when molds develop.

Potassium iodide reagent B (for bismuth determination), 16 grams of C.P. potassium iodide and 20 grams of ascorbic acid dissolved in distilled water to make 1 liter. This reagent must be prepared freshly about once each week.

Perchloric acid–nitric acid mixture, 3 volumes of 70% perchloric acid mixed with 1 volume of concentrated nitric acid.

Hydrogen peroxide, 30% solution.

STANDARD SOLUTION

Dissolve 110 mg. of C.P. potassium antimony tartrate in 500 ml. of distilled water, add 160 ml. of concentrated sulfuric acid, cool, and dilute to 1 liter with distilled water. One milliliter of this solution contains 40 micrograms of antimony. Fredrick has shown that the C.P. tartar emetics supplied by Mallinckrodt, Merck, and J. T. Baker are of the theoretical antimony content.

PROCEDURE FOR COLORIMETER

Measure into test tubes (preferably graduated at 10 ml.) quantities of standard solution ranging from 0.5 to 5 ml. Add sufficient 16 volumes % sulfuric acid to bring the volume of each solution to 5 ml. Then add 5 ml. of the potassium iodide reagent A, mix, and read in the colorimeter after 5 minutes (the readings remain constant for as much as 48 hours), using a filter having maximum transmission at 420 mμ wave length. To make the zero adjustment a solution consisting of a mixture of equal parts of 16 volumes % sulfuric acid and of potassium iodide reagent A is used. The readings which are obtained in this way constitute a calibration curve covering the range of 0 to 200 micrograms of antimony. However, readings up to 500 micrograms may be made.

The calibration curve is a straight line when the amount of antimony taken is between 50 and 500 micrograms. For amounts less than 50 micrograms there is a positive deviation—that is, the color obtained exceeds in intensity that which would be expected if Beer's law applied exactly—but the deviation does not exceed 5%. The region from 0 to 50 micrograms must therefore be calibrated specially if needed. A spectral-absorption curve

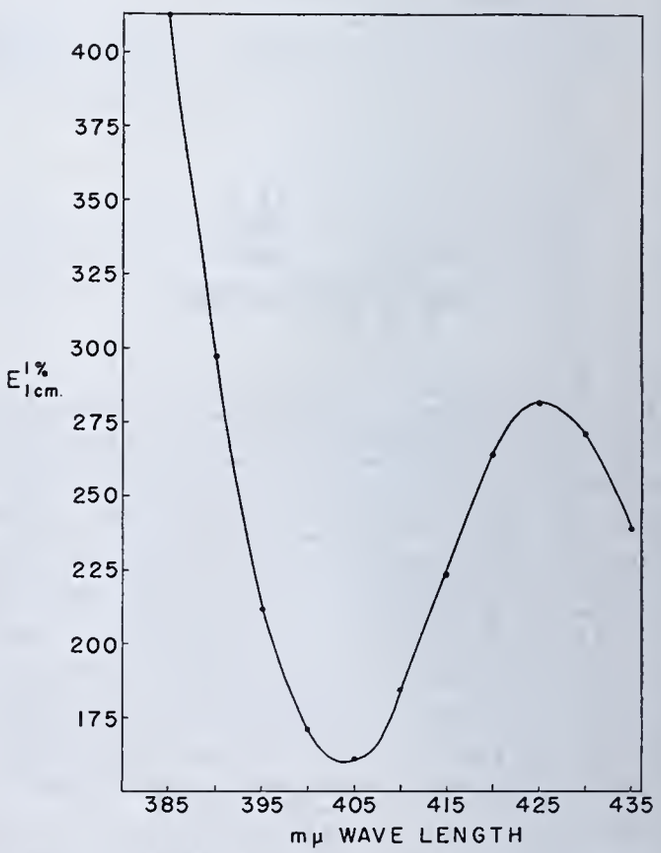

Figure 1. Partial Spectral-Absorption Curve of Potassium Iodoantimonite

Concentration expressed in terms of antimony

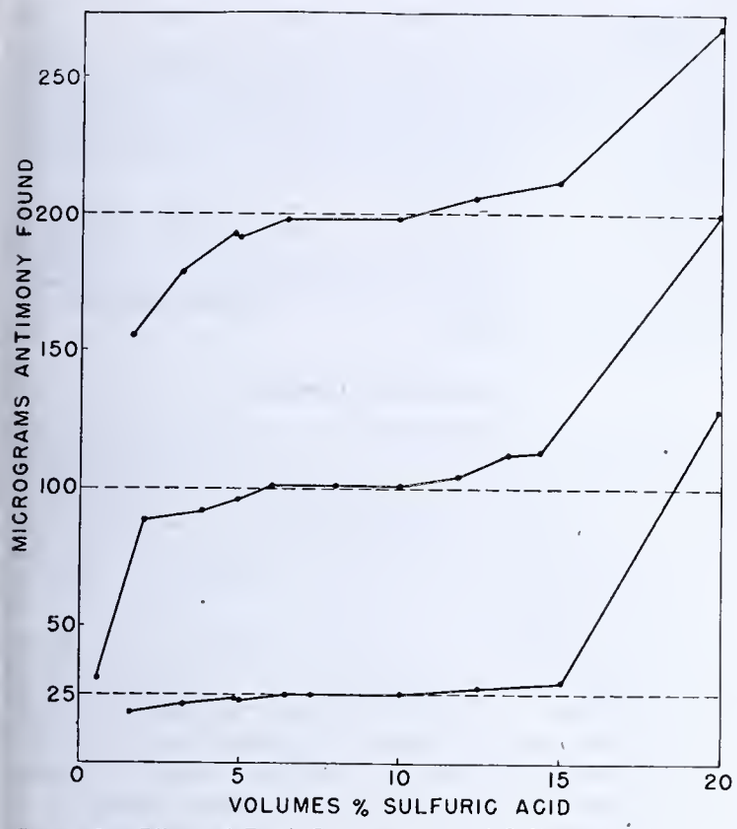

Figure 2. Effect of Final Concentration of Sulfuric Acid on Intensity of Color of Potassium Iodoantimonite Complex at Three Different Antimony Levels

for the colored complex is given in Figure 1. The maximum absorption is at 425 mμ; therefore the usual 42 filter may be used.

Sensitivity. The per cent transmission at 420 mμ of a solution containing 10 micrograms of antimony (in 5 ml. of 16 volumes % sulfuric acid, with 5 ml. of potassium iodide reagent A added) was 90.4 at a band width of 0.6 to 0.7 mμ. Thus 10 micrograms is about the smallest amount which one could hope to determine with any degree of accuracy; the observed standard error of a series of 6 estimations of 10 micrograms was 0.44 microgram, or 4.4%. When a Klett-Summerson instrument is available, the sensitivity may be approximately doubled by using the special absorption cell described by Giacomino (*5*). This would require, however, the establishment of another calibration curve for quantities of 0 to 50 micrograms.

EFFECT OF ACID CONCENTRATION

The color of the potassium iodoantimonite complex is materially influenced by the concentration of sulfuric acid, as is shown in Figure 2. The optimal concentration for quantitative work would appear to be 8 volumes %—that is, 16 volumes % in the acid digest of biological material—since over the range 6.3 to 9.7 volumes % there is no change in the intensity of color of the antimony complex. In fact, such extreme concentrations as 5 and 13 volumes % alter the color only to 95 and 105%, respectively, of that obtained at 8 volumes % concentration. A similar situation in the case of potassium iodobismuthite has been noted by Sproull and Gettler (*7*).

The acid concentration is kept within the desired range by the simple expedient of starting the digestion process within an amount of sulfuric acid which exceeds slightly that required to give 16 volumes % solution when finally diluted—for example, 4.2 ml. of concentrated sulfuric acid when diluted to 25 ml. gives a 16.7 volumes % solution. This amount of acid is therefore taken where a final dilution to 25 ml. is planned, and makes allowance for a loss of 0.2 ml. of acid during the digestion process. If a dilution to 10 ml. is planned, only 1.8 ml. of sulfuric acid are used for the digestion. This, again, makes allowance for a loss of 0.2 ml. Actual titrations of a number of digests have shown them to fall within the range 14.8 to 16.7 volumes %. It is obvious that excessive boiling of the digest, after the water has been driven off, must be avoided. For very accurate work the digests should be checked for sulfuric acid concentration unless the antimony concentration permits their dilution with known 16 volumes % acid.

EFFECT OF IODIDE CONCENTRATION

To achieve a concentration of potassium iodide equivalent to that used by Fauchon would require a reagent containing 16% of the salt—that is, 8% in the solution used for colorimetry. This concentration does, in fact, appear to give the maximum sensitivity (Figure 3, *A*). However, when the concentration is reduced stepwise the interesting fact is revealed that the antimony color begins to diminish rapidly, and at low concentrations disappears entirely. At the same time the bismuth color, which is also maximal when the reagent contains 16% of potassium iodide, loses only about 10% of its intensity (Figure 3, *B*). When the reagent contains 1.6% of potassium iodide, antimony gives no color whatsoever, and bismuth may be determined by what is essentially the Sproull and Gettler method. At 11.2% of potassium iodide the bismuth and antimony colors are exactly additive (Figure 3, *C*) and this has furnished a basis for the determination of both elements on the same digest.

METHODS OF ANALYSIS FOR ANTIMONY

Blood Plasma. Ten milliliters or less are measured accurately into a 100-ml. Kjeldahl flask, followed by 1.8 ml. of concentrated sulfuric acid and 5 ml. of a 3 to 1 perchloric-nitric acid mixture. Digestion is now carried out over a low flame, with addition of more of the oxidizing solution from time to time in order to prevent carbonization. When the oxidation is complete the digest is cooled, and 30 ml. of water are added and boiled off (to the appearance of fumes of sulfur trioxide). The digest is now accurately diluted to 10 ml. (For this operation, add 3 ml. of water to the digest and cool under the tap. Transfer quantitatively to a test tube graduated at 10 ml., using a 3-ml. pipet for the transfer. Rinse the sides of the flask with 2 ml. of water and transfer to the test tube. Repeat, then dilute with water to the mark.) To an aliquot an equal volume of potassium iodide reagent A is added. (A precipitate of potassium perchlorate may appear at this point. If so, it is centrifuged out.)

If the color obtained is beyond the readable range, the digest is diluted accurately with 16 volumes % sulfuric acid until a readable value is obtained. For a blank, the mixture of equal volumes of 16 volumes % sulfuric acid and potassium iodide reagent A is used. However, the digestion of a similar amount of antimony-free serum will give a small reading, which must be subtracted if accurate results are to be obtained on very low antimony concentrations.

Urine. Measure accurately into a 100-ml. Kjeldahl flask 25 ml. of urine and 4.2 ml. of concentrated sulfuric acid. Add approximately 10 cc. of concentrated nitric acid and boil gently over a small flame. Add more nitric acid from time to time to avoid carbonization. (The purpose of this is to avoid reduction of Sb^{V} to Sb^{III} in the presence of chloride, since antimony trichloride is slightly volatile.) When the oxidation is complete the digest is cooled and about 50 ml. of water are added and boiled off. At this stage the digest should not have any yellow color; if it does,

Table I. Interference of Some Metallic Elements with Determination of Antimony[a] as $KSbI_4$

Element Added	Amount Added	Antimony Found
	Micrograms	*Micrograms*
As^{III}	100	100
Hg^{II}	100	100
Bi^{III}	100	242
Fe^{II}	200	101
Cu^{II}	100	100
Pb^{II}[b]	200	102
W^{VI}[b]	200	99
Tl^{I}[b]	200	82[c]

[a] 100 micrograms.
[b] These elements were included at the suggestion of W. G. Fredrick. Tungsten and thallium interfere with the rhodamine B procedure for antimony.
[c] Forms a yellow precipitate which can be removed by centrifugation. Apparently some potassium iodoantimonite is adsorbed on precipitate.

Table II. Recovery of Antimony Added to Biological Materials

Material	Antimony Added[a]	Antimony Recovered[b], (Mean)	Standard Error of Mean[c]
	Micrograms	*Micrograms*	%
Beef blood plasma, 10 ml.	0	3	....
	12.5	12.7	13.5
	100	99	2.9
	500	516	1.7
	1250	1275	2.1
Human urine, 25 ml.	0	10	....
	250	255	2.2
	750	728	1.7
	1250	1253	2.7
	5000	5040	0.7
	1765[d]	1770	1.4
	2360[e]	2300	1.8
Dry rabbit feces, 0.5 gram	0	3	....
	250	249	3.5
	2500	2544	1.3
Rabbit liver (fresh), 2.5 grams	0	0	....
	250	244	2.0
	2500	2450	1.2

[a] As tartar emetic except where otherwise indicated.
[b] After subtraction of established blank.
[c] From mean.
[d] As Neostibosan, a preparation containing *p*-aminophenylstibonic acid, its acetyl derivative, and antimonic acid.
[e] As Stibanose, a preparation containing the complex of sodium gluconate and antimonic acid as the diethyl monoethanolamine salt. The antimony analysis of this compound and of Neostibosan was kindly supplied by H. B. Corbitt of the Winthrop Laboratories.

a few drops of Superoxol or of nitric-perchloric acid mixture are added to the hot solution and the boiling is continued for another minute. The digest is now diluted to 25 ml. and a 5-ml. portion (which contains about 16 volumes % sulfuric acid) is taken for colorimetric analysis as for blood plasma. If the amount of antimony exceeds that which can be read, the digest is diluted with 16 volumes % sulfuric acid until a readable color is obtained. (Or the colored solution may simply be diluted with the sulfuric acid–potassium iodide solution—which is used for the blank—until the color is within the readable range.) A blank determination on 25 ml. of antimony-free urine must be run and subtracted, particularly if the amount of antimony to be determined is very small.

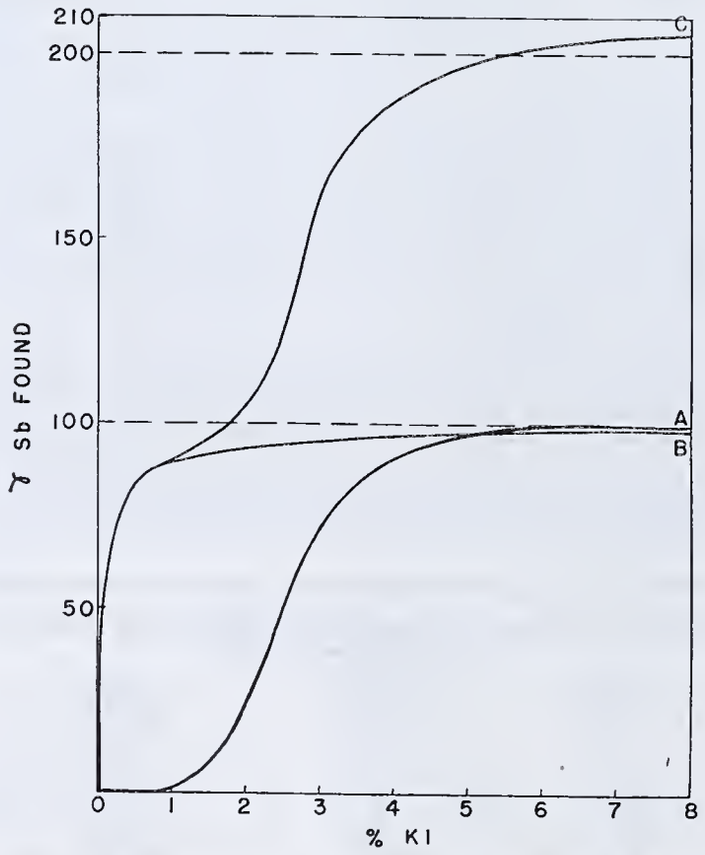

Figure 3. Effect of Final Concentration of Potassium Iodide on Intensity of Color

A. 100 micrograms of antimony
B. 70 micrograms of bismuth
C. 100 micrograms of antimony and 70 micrograms of bismuth

In all cases 100 is taken as the amount of color obtained when 100 micrograms of antimony in 5 ml. of 16 volume % sulfuric acid are mixed with 5 ml. of a solution containing 16% potassium iodide and 2% ascorbic acid

FECES. Samples of 0.5 gram of dry feces, or about 2 grams of wet feces, are transferred to a 100-ml. Kjeldahl flask followed by 4.2 ml. of concentrated sulfuric acid and 10 ml. of concentrated nitric acid. The procedure is as for urine. The blank value for antimony-free feces is negligible unless very small amounts of antimony are being determined (see Table II).

TISSUES. A sample expected to contain 250 micrograms of antimony or more is transferred to a 100-ml. Kjeldahl flask along with 4.2 ml. of concentrated sulfuric acid. The oxidation process is carried out with perchloric acid–nitric acid mixture; otherwise the process is as described for urine. The blank value for antimony-free tissue is negligible.

INTERFERING ELEMENTS

The very low blank values obtained with the various body tissues and excreta make it clear that none of the metallic elements normally found in the body interferes with the antimony determination. Interference might be expected, however, from some of the heavy metals which could enter the organism in the form of various medications. Results covering some of these elements are given in Table I.

The only seriously interfering element is bismuth. A method is described below by which this element may be determined separately, if present. As for anions, there is no interference from nitrates or phosphates. Oxidizing anions such as nitrite and hypochlorite interfere if present in sufficient quantity to oxidize the ascorbic acid in the potassium iodide reagent. Sulfites interfere in any concentration, since they give a yellow color when added to a reagent blank.

RECOVERY OF ANTIMONY FROM BIOLOGICAL MATERIALS

The results of recovery experiments are given in Table II. The values obtained are satisfactorily precise, as is shown by the standard errors. As a matter of fact, these errors are ordinarily no greater than would be obtained in setting up and reading a series of half a dozen standards of the same antimony concentration. The recovery experiments have included several forms of the element. In most cases the antimony was added as tartar emetic, but in some cases organic compounds such as Neostibosan and Stibanose were added.

METHOD FOR DETERMINING BOTH ANTIMONY AND BISMUTH

The colorimeter must be accurately calibrated for bismuth using both the 1.6 and 11.2% potassium iodide reagents. A given amount of bismuth yields 11% more color at the higher iodide concentration.

An aliquot of the digest is treated with an equal volume of reagent B and another aliquot is treated with an equal volume of reagent A. Both are read in the colorimeter against a blank consisting of one volume of 16 volumes % sulfuric acid and one volume of potassium iodide reagent A. The first aliquot gives the amount of bismuth present, and the second gives antimony plus bismuth. If the second reading is beyond the range of the instrument, the digest is diluted with 16 volumes % sulfuric acid until a readable color can be obtained with reagent A, then both determinations are repeated.

Table III. Recovery of Antimony and Bismuth from Biological Materials

Material	Bismuth Added[a]	Antimony Added[b]	Bismuth Recovered ± Standard Error	Antimony Recovered ± Standard Error
	Micrograms		*Micrograms*	
Human urine, 25 ml.	600	600	594 ± 29.0	617 ± 26.0
	1000	200	981 ± 8.6	214 ± 17.0
	200	1000	214 ± 10.5	986 ± 14.0
	200	200	205 ± 6.5	201 ± 6.0
Beef blood plasma, 10 ml.	50	50	48.7 ± 1.8	49.2 ± 3.3
	100	100	96.5 ± 3.0	104.0 ± 5.3
	200	200	203 ± 3.5	204.5 ± 3.9

[a] As bismuth nitrate.
[b] As potassium antimony tartrate.

CALCULATION. The bismuth content is calculated from the established calibration curve for reagent B. The colorimeter reading at the lower iodide concentration is then multiplied by 1.11 and subtracted from the reading at the higher iodide concentration. The difference is due to antimony and is calculated in the usual manner from its calibration curve. Some recovery experiments involving both elements are given in Table III. The total recovered is more accurate than either element alone; an accuracy better than ±5% is not claimed for the individual elements.

DISCUSSION OF METHOD

The ascorbic acid which is present in the potassium iodide reagent serves three purposes: (1) it reduces the iodine formed by the reaction $Sb^{V} + 2KI \rightarrow Sb^{III} + I_2 + 2K^{+}$; (2) it stabilizes the color by preventing the formation of free iodine from potassium iodide; (3) it acts as a buffer against traces of oxidizing impurities which may be present in the digests. These properties of ascorbic acid eliminate the necessity for any elaborate process for the destruction of oxidizing impurities.

One important source of error is the presence of yellow substances in the acid digests. These may appear to have been completely destroyed and be invisible to the naked eye. Yet when the amount of antimony present is small and large aliquots of the digest must be used, the error may be appreciable. It is advisable as a precaution to read the color of the digest in the colorimeter against 16 volumes % sulfuric acid and to make a correction appropriate to the aliquot used. If the aliquot used is as little as 1 ml. this factor is usually negligible.

The only other technical points which require attention—namely, control of acid concentration, and avoidance of loss of antimony as antimony trichloride—have been sufficiently emphasized.

ACKNOWLEDGMENT

The author is indebted to W. G. Fredrick for a number of constructive suggestions which have added materially to the value of this paper, and to G. E. Ewing of the Winthrop Laboratories for making the measurements on the Beckman spectrophotometer.

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Microdetermination of Mercury in Biological Material

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A procedure suitable for determining minute quantities of mercury in biological and related materials is described. Organic matter is destroyed by digesting the sample in a special apparatus, by boiling first with a mixture of sulfuric and nitric acids, followed by a second boiling treatment after addition of potassium permanganate. Mercury is extracted from the decolorized acid-permanganate digest (diluted to 1.8 *N* in respect to sulfuric acid) by means of a chloroform solution of di-β-naphthylthiocarbazone. The chloroform extract is then treated with a sulfuric acid–sodium thiosulfate mixture to separate the mercury from copper. The final estimation of mercury is made by photometering di-β-naphthylthiocarbazone–chloroform extracts of the copper-free aqueous phase following treatment with potassium permanganate. At least 50 grams of blood must be used for the reliable estimation of concentrations as low as 1 microgram of mercury in 100 grams of blood. A table, which compares the mercury content of the blood and urine of exposed persons, shows that the mercury content of the blood is very low. Except in one case, the amounts were well below 10 micrograms for 100 grams of blood. The highest, 0.029 mg. per 100 grams, was found in a case where the urine level was 4.44 mg. of mercury per liter. The mercury–di-β-naphthylthiocarbazone complex is more stable than the analogous dithizone complex.

SATISFACTORY methods for the determination of minute quantities of mercury, particularly in the air of industrial establishments and in the urine of exposed persons, have been described (*3*, *4*, *8*, *11*, *12*). The most sensitive of these methods involving the scattering of the radiation at 2537 Å. by mercury vapors (*3*, *12*) is very satisfactory for air analysis, but its application in the analysis of other material requires special equipment and inconvenient preparatory procedures. Ballard and Thornton (*1*) have applied the method to the determination of minute quantities of mercury in aqueous solutions and have mentioned the possibility of its use in the analysis of biological material, but have given no data or details of procedure on such applications. Laug and Nelson (*6*) have described a simple chemical method based upon the use of dithizone for the general analysis of biological material, but the authors were unable to apply the method successfully whenever it was necessary to employ all of a large sample. Others (*10*) also have reported difficulties in the use of the Laug and Nelson method.

The colorimetric method founded upon the use of di-β-naphthylthiocarbazone (*4*) has been employed successfully by the authors in the analysis of samples of air, urine, and aqueous solutions of organic materials which can be oxidized conveniently by digestion with acid–potassium permanganate. In applying the method to the analysis of other material, however (solid tissues, blood, etc.), difficulties were encountered mainly because of losses of mercury due to the methods used in oxidizing the organic matrix. The general applicability of the method depended therefore upon the development of safe oxidation procedures and upon the introduction of modifications in the extraction steps which would eliminate the need for using small samples or aliquots in order to protect the mercury extraction agent. Such a convenient procedure has been developed and is given below in such detail as to involve some slight repetition of earlier published work (*4*) for the sake of completeness.

REAGENTS

Oxidizing Mixture. A 1 to 1 mixture of concentrated sulfuric and nitric acids is convenient to use for the destruction of organic matter.

Hydroxylamine Hydrochloride, 50 and 5% w/v solutions.

Potassium Permanganate, 5-grain (0.325-gram) tablets and a saturated solution.

Sodium Thiosulfate, 1.5 grams of sodium thiosulfate in 100 ml. of water.

Di-β-naphthylthiocarbazone. (1) Initial extraction solution, 20 mg. of di-β-naphthylthiocarbazone in 1 liter of chloroform. (2) Final extraction solutions, 0 to 5 micrograms range 6 mg. of di-β-naphthylthiocarbazone per 1 liter of chloroform; 0 to 50 micrograms range same as initial extraction solution.

The extraction solutions are stabilized and used chloroform is reclaimed by procedures described by Bambach and Burkey (*2*).

PROCEDURE

Preparation of Samples. Urine (and aqueous solutions of most organic materials). Fifty-milliliter samples are digested with sulfuric acid and potassium permanganate in a boiling flask provided with a "cold finger" as previously described (*4*).

Air Samples. Atmospheres may be sampled either by passing the air at a suitable rate through a gas scrubber containing 100 ml. of a solution of potassium permanganate (10 grams) in 10% (v/v) sulfuric acid, or the sample may be collected in an evacuated balloon flask (*7*) and the mercury brought into solution by introducing 100 ml. of the absorbent medium into the flask and shaking it for a few minutes. The entire sample or a suitable aliquot may be used, but only after decolorizing the solution with hydroxylamine hydrochloride solution (50% w/v).

Solid Tissue and Blood. Introduce the sample (1 to 50 grams) into a 1-liter, two-necked, distilling flask (Figure 1), add 40 ml. of the oxidizing mixture, insert the cold finger, and heat the mixture to boiling. After boiling for 2 hours, cool, then further chill the solution by immersing the flask in an ice bath. Remove the fats and fatty acids which separate out, by filtering the solution through a glass-wool plug inserted into the neck of a funnel. Collect the clear filtrate in a second 1-liter, two-necked flask, reinsert the finger, add two 5-grain tablets of potassium permanganate, and again heat the solution to boiling. Continue boiling and add tablets of potassium permanganate (2 at a time), through the second neck of the flask, either until a precipitate of manganese dioxide persists or until 8 to 10 tablets have been used for each 10 grams of sample. Cool the flask again in an ice bath and add 5 ml. of hydroxylamine hydrochloride solution (50% w/v) to decolorize the solution. If, on cooling, fats again separate out, filter the solution as after the first chilling.

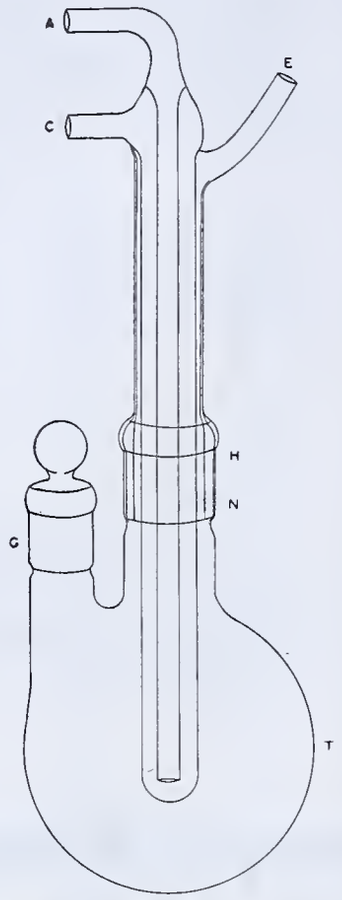

Figure 1. Digestion Apparatus Used in Oxidation of Organic Material

Height of apparatus, 40 cm.

A. Water inlet
C. Water outlet
E. Gas outlet
G. $\frac{24}{40}$ ♀
H. $\frac{34}{45}$ ♀
N. 1.5- to 2-mm. gas gap
T. 1-liter Pyrex flask

Initial Extraction. Proceed with the extraction of mercury from samples of digested urine as directed previously (*4*). Solutions resulting from air sampling, containing not more than 10% (v/v) sulfuric acid, may be handled in a similar fashion. In all cases in which 40 ml. of the oxidizing mixture have been used, transfer the prepared sample to a 500-ml., pear-shaped, separatory funnel, dilute it to 400 ml. with distilled water, add 5 ml. of hydroxylamine hydrochloride solution (50% w/v), and extract the mercury with 5-ml. portions of initial extraction di-β-naphthylthiocarbazone solution. Continue extraction with 5-ml. portions of di-β-naphthylthiocarbazone solution until the last portion added shows no change in color, collecting the extracts in a second 150-ml. Squibb separatory funnel. (Each 5-ml. portion of di-β-naphthylthiocarbazone solution extracts approximately 25 micrograms of mercury and the total quantity of di-β-naphthylthiocarbazone used indicates the concentration range, thereby determining the final dilution of the sample and the size of the cell which is to be used for photometric purposes. If minute traces of mercury are expected, the individual portions of extracting solution used may be reduced to 1 or 2 ml. in volume in order to make more certain by the color change that less than 5 micrograms of mercury are present.)

To the combined chloroform extracts or to an aliquot containing not in excess of 50 micrograms of mercury in the second funnel, add 50 ml. of dilute sulfuric acid (2% v/v) and 4 ml. of the sodium thiosulfate solution. Shake for 1 minute and discard the chloroform layer. Wash the aqueous solution twice with 5-ml. portions of clear chloroform and then transfer the aqueous laye to a 200-ml. boiling flask. Add 5 ml. of saturated potassiur permanganate solution, insert a cold finger in the mouth of th flask (*4*), and heat the solution for 10 minutes. Cool the solu tion and decolorize it by adding aqueous hydroxylamine hydro chloride (5% w/v) dropwise, and then add 1 ml. in excess. Re insert the condenser and heat just to the boiling point. Cool an rinse the solution into a 150-ml. Squibb-type separatory funnel

Final Colorimetric Extraction. Dilute the solution to 10 ml. and extract the mercury with di-β-naphthylthiocarbazon solution, using 20 ml. of the initial extraction solution for th range 0 to 50 micrograms, or 10 ml. of the 6 mg. per liter solutio for the 0 to 5 micrograms range. For photometric readings in th lower range of concentrations a cell of 50-mm. light path is em ployed, while for the higher a cell of 10-mm. light path is satis factory. Any suitable photometer capable of using Style I American Instrument Company cells may be used. Filters fo photometers that require them should be centered at 515 mμ.

Working curves are obtained with known quantities of mer cury (as the nitrate) plus 50 ml. of sulfuric acid (2% v/v) an 1 ml. of hydroxylamine hydrochloride solution (5% w/v) di luted to 100 ml. as in the final colorimetric extraction step.

RESULTS

The recoveries in the case of known quantities of mercur added to blood and tissue samples (liver) are listed in Tables and II. Table III gives some results obtained upon samples o the blood and urine of exposed persons in order to illustrate th relationships between the blood and the urine with respect t concentration of mercury. These data demonstrate the practica need for a micromethod capable of using such relatively larg samples.

DISCUSSION

As is indicated in Table I, it is possible to detect as little as 0.5 microgram of mercury in 50 grams of blood, this being the small est quantity of sample that can be employed successfully whe the concentration of mercury is of the order of 1 microgram pe 100 grams. Mercury cannot be extracted from acid digests o such large samples, prepared by the Laug and Nelson metho (*6*), either because of the large quantity of nitric acid which mus be used or because in the oxidation of the organic matter certai products are produced which destroy the extracting agent (di

Table I. Recoveries of Mercury Added to Blood Samples

Blood Grams	Tablets of $KMnO_4$ Employed	Mercury Added Micrograms	Mercury Found Micrograms
50	40	0	1, 0.8 (0.9 av.)
20	20	0	0.7, 0.5 (0.6 av.)
50	40	0.5	1.4, 1.4
50	40	1.0	1.6, 2.0
50	40	5	5.9, 5.3
50	40	10	11, 11
50	40	20	21, 20
20	20	50	50, 50.5
20	20	500	505, 495

Table II. Recoveries of Mercury Added to Rabbit Liver

Liver Used Grams	Tablets of $KMnO_4$ Employed	Mercury Added Micrograms	Mercury Found Micrograms
50	40	0	3.8, 2.6 (3.2 av.)
50	40	5	8.4, 8.4
50	40	100	101, 102.5

Table III. Concentration of Mercury in Blood and Urine of Exposed Persons

Number	Urine	Blood	
	Mg./l.	Grams	Mg./100 g.
J.S.	0.12	55.6	0.001
W.H.	1.06	50.3	0.009
L.P.	1.20	33.5	0.006
O.M.	0.82	33.4	0.003
L.W.	0.04	58.5	0.001
H.M.	4.44	47.7	0.029
M.B.	0.64	32.4	0.001
J.W.	0.18	53.7	0.001

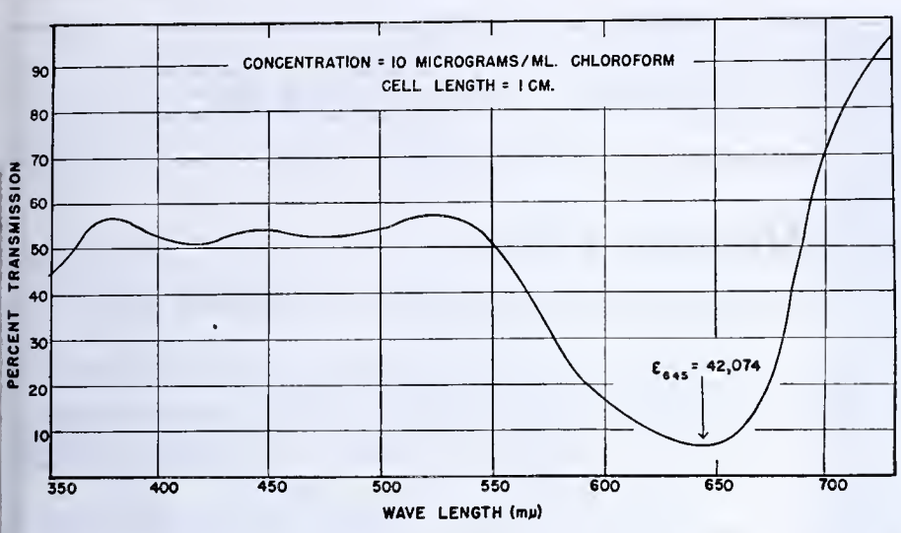

Figure 2. Transmission Curve for Twice Repurified Di-β-naphthylthiocarbazone in Chloroform

thizone or di-β-naphthylthiocarbazone). The use of potassium permanganate in the authors' method overcomes these difficulties, for not only does this additional treatment destroy more of the organic matter, but in the process of doing so it neutralizes some of the acid (probably much of the nitric acid) and permits extraction of the mercury without decomposition of the extracting agent. The use of the additional reagent increases the magnitude of the reagent blank, but not to such an extent as to be practically disadvantageous. The total blank associated with the employment of 40 ml. of the standard oxidizing mixture has been found to be 0.9 and 0.6 microgram, respectively, when 40 and 20 tablets of potassium permanganate are used.

The dilution of the prepared sample to the volume of 400 ml. also tends to prevent oxidation of the di-β-naphthylthiocarbazone and reduces the concentration of acid therein (in terms of sulfuric acid employed) to about 1.8 *N*, which is of the order of that (1.5 *N*) used by Laug and Nelson (*6*), in the initial extraction. The authors have not found it necessary to prevent the extraction of silver by the addition of hydrochloric acid, since this is accomplished by hydroxylamine hydrochloride. Added quantities of silver up to 1 mg. carried through the complete procedure failed to affect the recovery of mercury.

The removal of free fats or fatty acids is necessary because of their effect on the extraction agent, but their removal, as also shown by Laug and Nelson (*6*), does not result in losses of mercury. Experience has demonstrated that some fatty material is dissolved by the chloroform in the initial extraction step and that this may be carried over into the acid-thiosulfate mixture. A considerable portion of this fat is removed when the acid-thiosulfate mixture is washed with clear chloroform and the greater portion of the remainder is destroyed in the acid-permanganate boiling procedure used to break up the mercury-thiosulfate complex preparatory to the final colorimetric extraction. A slight yellow color remains in the aqueous layer (particularly when large samples have been employed), but this color does not appear to be extracted by the final extraction solution.

The use of the thiosulfate complexing medium permits a final extraction for colorimetric purposes at pH 0.5. This is beneficial in avoiding interference by other metals, particularly lead, zinc, and cadmium, which might prove harmful at pH 6, such as is used by Laug and Nelson (*6*), particularly if through error the acidity should be less than this value. Laug and Nelson avoid these possible interferences by purifying their iodide and buffer solutions, steps which are unnecessary if the method described above is used. The only elements that might be expected to give trouble under the conditions of extracting at relatively high acidity are copper, silver, and bismuth. The latter two, however, are not extracted in the presence of chloride ion (obtained from the hydroxylamine hydrochloride which is added in both the initial and final extraction steps), while the former remains in the chloroform layer when the initial extraction solution is shaken with the acid-thiosulfate solution. In the use of the latter the only precaution required is that the acid-thiosulfate solution should be made up fresh just before use; otherwise the thiosulfuric acid is decomposed with deposition of sulfur and consequent loss in mercury-complexing ability.

The lack of stability of the mercury-dithizone complex (*6*) is a decided disadvantage which has been overcome by the use of di-β-naphthylthiocarbazone. The complex of the latter reagent with mercury is stable and no loss in color has been observed after 1 hour of exposure of the solutions to strong artificial light. No effort is made to protect the solutions of the mercury-di-β-naphthylthiocarbazone complex prior to their final introduction into the photometer, although they are not allowed to stand in strong sunlight.

The di-β-naphthylthiocarbazone was prepared by one of the authors, by a method described elsewhere (*5*). The reagent may be obtained from Eastman Kodak Corporation, but the product so available at present must be purified, since its molecular extinction coefficient (ϵ) at 645 mμ was found to be approximately 10,000 as compared to that of 42,000 which characterizes the twice repurified product made by the Scott and Hubbard method and which was used in this work (*5*). After one purification, the Eastman product had an extinction coefficient of 25,000. Apparently the method originally used by Hubbard (*4*), which was a modification of Suprunovich's method (*9*), gave a somewhat purer product than that made subsequently (*5*), since an extinction coefficient of 45,700 was obtained for it. This apparently superior method, however, is impractical because of its very low yield. In Figure 2 is shown a transmission curve for the twice purified product made by the Hubbard and Scott method (*5*). This curve was obtained with the Beckman spectrophotometer, the readings being made at 10-mμ intervals until the peak of absorption was near, when they were made at 5-mμ intervals. By reason of the use of the Beckman instrument, this curve is more accurate than that shown previously (*4*).

Finally, the authors have attempted to employ the spectrograph for the determination of mercury in biological material. The spectrographic sensitivity was found to be satisfactory only following isolation of the mercury and reduction of the resultant solution to a volume of 0.5 or 1 ml. These procedures always resulted in some loss of mercury and therefore the spectrographic method was abandoned in favor of the purely chemical method described above.

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A Versatile Electronic Relay

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An electronic relay is described which is variable in sensitivity, requires at most only a few microamperes of contact current, can be adjusted to the inherent resistance of the regulator circuit, and incorporates a photocell for light activation of the relay.

A NUMBER of electronic relay circuits have been described in the literature (*1*, *3–6*) during the past few years. The general trend has been toward the development of a relay requiring regulator contact current in the order of a few microamperes, so as to prevent deterioration of the mercury-platinum or the bimetallic strip regulator contacts. In using these relays, it is necessary that the regulator elements be well insulated and their connecting leads short and with good insulation, since any reduction in the necessarily high open circuit resistance, due to high humidity or insulation leakage, may prevent normal use of the relays. Moreover, these relays are usually of fixed types, each serving only one purpose, so that, for different uses, either several specific types of relays must be present in the laboratory or circuit changes in the existing instruments must be made.

The relay described in this paper offers the following features not found as a group in the usual electronic relay:

By means of a six-point selector switch in conjunction with a potentiometer, the relay can be adjusted rapidly for use with any type of regulator having contact and connecting wire lead resistances from approximately 0 ohm to 250 megohms.

It is provided with a socket to receive an emission-type photocell for light activation of the relay, to operate either alone or in conjunction with the usual type of regulator.

The relay will open or close the 117-volt alternating current control contacts either upon opening or closing of the regulator contacts, or with increase, decrease, or interruptions in the light striking the photocell.

Using the low resistance range, the regulator contact current does not exceed a few microamperes and is as low as a fraction of a microampere for photocell operation or in using the highest resistance range.

Series resistances are provided to protect the operator from accidental shock by body contact with the regulator terminals.

RELAY CIRCUIT

The relay circuit shown in Figure 1 illustrates the use of a type 2050 gas tetrode (interchangeable with type 2051), operating on alternate half waves of the alternating current cycle, in a conventional circuit (*2*) modified for the purpose. This type of tube was chosen for its high current-carrying capacity and for the triggering action of the grid where, when the critical grid-firing voltage is reached, the tube conducts to its maximum capacity. The necessary voltages for plate and grid operation are obtained from the voltage divider, R_1 to R_4, with the voltage developed across R_1 and R_2 supplying the plate a voltage positive with respect to the cathode, and the voltage across R_3 and R_4 negative with respect to the cathode on the same half cycle for grid control. R_3 gives a minimum negative grid bias voltage just in excess of cutoff, while R_4 allows for an additional grid bias voltage, variable to the grid through potentiometer R_5. The use of resistors R_8 to R_{13} allows for the ranges of sensitivity desired for operation of the relay.

With S_2 in position 6 and the control on R_5 moved all the way up (minimum setting), the relay will operate with contact resistances across T_R of as much as 250 megohms, while with S_2 in position 1 and the control of R_5 all the way down (maximum setting) practically a zero resistance is necessary. On adjusting S_2 to intermediate contact points and varying R_5 for each position of S_2, a complete range in between is available.

The plate circuit relay, R_E, is of 1500 ohms' resistance requiring approximately 8 ma. for operation. Under the conditions of available plate voltage, a relay requiring up to 35 ma. and of lower resistance could have been used. However, under these conditions and especially if the circuit is to be turned off and on frequently, a switch should be inserted in the plate circuit to provide a minimum delay of 10 seconds after turning on S_1 before applying the voltage to the plate of the tube. This is recommended by the manufacturer of the tube, since turning on the heater and plate voltages at the same time may result in reduced tube life. The authors have not experienced this difficulty in the use of the instrument as described. The holding condenser, C, is necessary to prevent chattering of the plate circuit relay, since the operation of the relay depends upon half-wave plate rectification. The plate circuit relay has a set of single-pole single-throw contacts connected to a pilot lamp to indicate operation and a set of single-pole double-throw contacts to deliver up to 6 amperes at 117 volts alternating current to a dual outlet receptacle, so that "normally open" or "normally closed" relay service is available for operation of the desired control device. If a greater load is to be operated, a heavy-duty alternating current relay can be placed in the control contact circuit or a plate circuit relay with suitable contacts can be substituted.

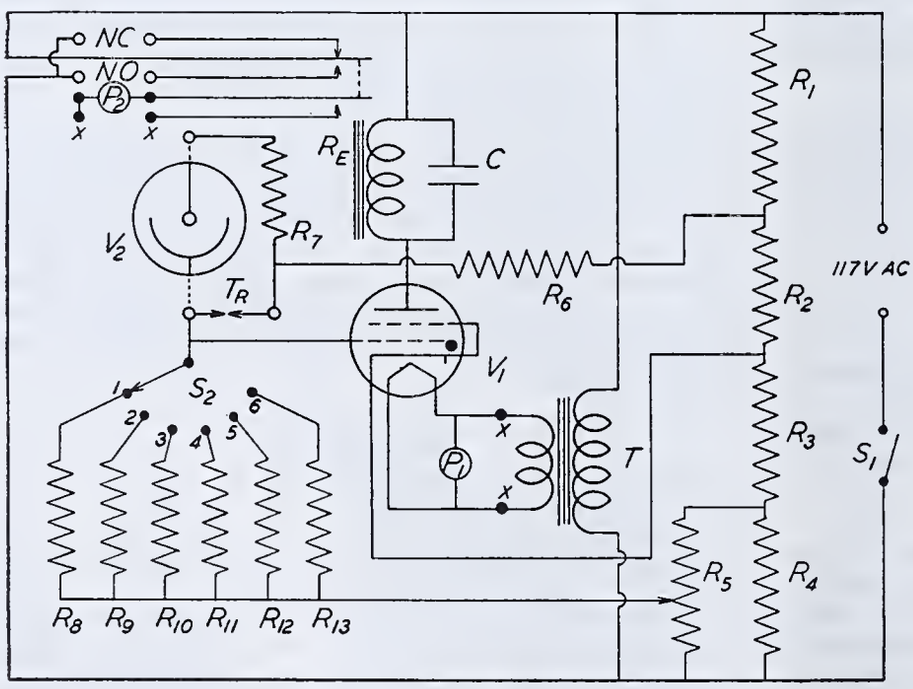

Figure 1. Circuit Diagram of Relay

V_1 = 2050 gas tetrode; V_2 = 868 gas photocell; T = midget filament transformer 117, 6.3-volt Thordarson T19F80; R_E = plate circuit relay; P_1, P_2 = 6.3-volt pilot lamps, Mazda No. 44, in Drake No. 20 jeweled sockets; S_1 = midget toggle switch; S_2 = six-position Mallory selector switch No. 1316L; C = 10-μfd. electrolytic condenser, 50-volt DCWV; R_1 = 10,000 ohms; R_2 = 5000 ohms; R_3 = 900 ohms; R_4 = 1750 ohms; R_5 = wire-wound potentiometer of 10,000 ohms, Mallory No. M10MP; R_6 = 250,000 ohms; R_7 = 5 megohms; R_8 = 100,000 ohms; R_9 = 250,000 ohms; R_{10} = 500,000 ohms; R_{11} = 1 megohm; R_{12} = 5 megohms; R_{13} = 10 megohms. Resistors R_1 to R_4 are Brown Devil 10-watt wire-wound resistors. R_6 to R_{13} are 0.5-watt carbon resistors. T_R = thermoregulator, *NC* and *NO* are normally closed and normally open relay contacts. Pilot lamp P_2 indicates when relay is energized, while P_1 indicates continually while instrument is in use

The transformer, T, is a midget filament transformer to supply 6.3 volts to the heater of the 2050 tube. Resistor R_7 is a current-limiting resistor for the type 868 photocell. R_6 is a regulator contact protective resistance and should be approximately 250,000 ohms. Figure 2 is a photograph of the instrument.

BASIC OPERATION OF THE CIRCUIT

The circuit operates in the following manner. With the regulator terminals, T_R, open or the phototube dark (or at some predetermined light intensity depending on the setting of S_2 and R_5), the grid of the 2050 is at a voltage more negative than the critical grid firing voltage as determined by R_3 and the setting of R_5. When contact T_R is closed, or the photocell illuminated (or the illumination increased), the grid is brought to a potential less negative than the cutoff voltage, causing the tube to fire and energize the plate circuit relay.

OPERATION OF THE RELAY

After connecting the relay to a thermoregulator or similar activating mechanism and with contacts T_R open, switch S_2 can be moved progressively from contact 1 toward contact 6 and if the relay operates, owing to leakage across the wire leads from the regulator, switch S_2 can be backed off one position (or the setting of R_5 lowered) until the plate circuit relay does not become energized except when contacts T_R are closed. Actually for most uses of the relay, except under conditions of very high humidity, or when using the photocell, switch S_2 can be left permanently in positions 3 or 4. For excitation of the relay using the photocell, the procedure described previously can be employed. When the position of S_2 is found where the relay operates for any given light intensity on the photocell, backing off S_2 one step will cause it to cease operation. A position of R_5 can then be found where slight movement one way will cause the relay to operate, and movement in the opposite direction will cause it to be nonenergized. If R_5 is now left in the position where the plate circuit relay does not operate, a slight increase in the light will cause it to be energized and with a decrease it will again be inoperative. The instrument is made very flexible in its use by simply adjusting S_2 and R_5 as outlined.

The relay is easy to assemble, requires no preliminary adjustments, and should cost approximately \$13.50 for all parts. If photocell operation of the relay is not desired, V_2 and R_7 can be omitted, reducing the total cost to \$10. The present relay has been in almost continuous use without any attention for more than two years, controlling the temperature of a constant-humidity chamber using a toluene-mercury-platinum thermoregulator.

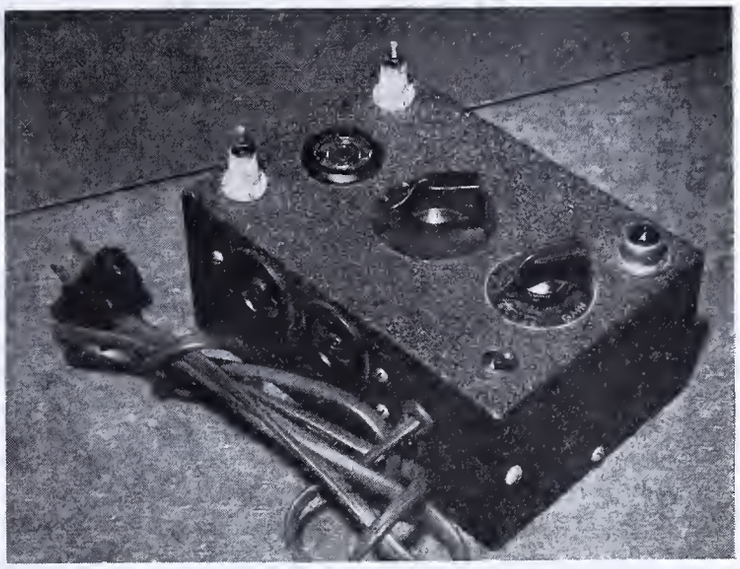

Figure 2. Photograph of Relay

Through-panel insulators for thermoregulator T_R are at far end of panel with photocell socket V_2 between them. Selector switch S_2 is in center and control to R_5 below. To left of control to R_5 is switch S_1; P_1 is to right. Pilot lamp P_2 is not visible from this view. Dual outlets to *NO* and *NC* are on left side of cabinet. Entire assembly is a $2^5/_8 \times 4^1/_8 \times 6^1/_8$ inch cabinet, black crackle finish

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Kettle for Measuring Small Residues in Low-Temperature Fractional Distillation

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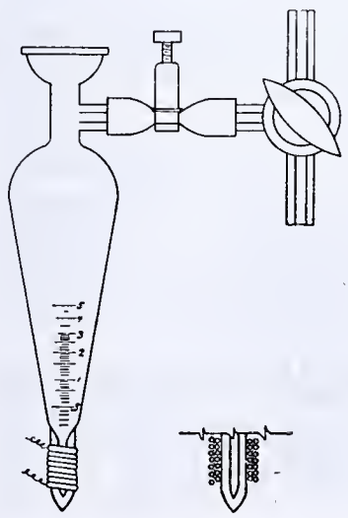
SOME samples for low-temperature fractional analyses require the measurement of very small liquid residues, when under the conditions of the analysis, it is impossible to vaporize the residue and measure it as a gas. If it is necessary to pour the residue from the kettle before measuring it, very large samples must be taken, so that the liquid remaining in the kettle will not be a large part of the total residue. A kettle in which it is possible to measure accurately small residues is advantageous, since time is saved by the use of samples of moderate size. Two types of sample which contain a very small proportion of heavy material are the effluent from a polymerization plant and the raw gasoline stream from a natural gasoline plant which recovers a large amount of propane and butane.

The figure shows a kettle made from the lower end of a California-type 100-ml. graduated centrifuge tube. The heating coil is wound upon a 19-mm. piece of capillary tube, which is fused to the end of the centrifuge tube. The volume of the capillary is determined by adding liquid from a buret to the centrifuge tube; if the buret reads 1.1 ml. and the centrifuge tube reads 1.0 ml., the volume of the capillary is 0.1 ml. The heater consists of 23 cm. of No. 28 resistance wire, which is wound between two layers of closely wound asbestos or glass string. The wire may be threaded through a Fiberglas sleeve before winding. The string and the wire are fixed by the liberal application of water glass.

The upper section of the centrifuge tube is pulled off at about the 30-ml. mark, and an 18/9 female ball joint is sealed to the rounded end. The male part of the joint is sealed to the distilling tube of the low-temperature fractionating column. The sample is introduced through the capillary connection which is sealed to the stem of the female joint. The three-way stopcock is attached to the capillary by means of a short piece of rubber tubing. A pinchclamp or a screwclamp on the rubber tubing helps to prevent leaks around the stopcock. The vertical arms of the cock are used to purge the line from the sample container. The kettle is immersed in liquid nitrogen contained in a Dewar flask during introduction of the sample.



After distillation is completed, the kettle is immersed in ice water, in order to condense vapor and cause the reflux liquid to leave the packing of the distilling tube and collect in the kettle. The kettle is then immersed in water at 60° F., and the volume of the residue is read.

Hot Stage for Microscopic Observations between Room Temperature and 350° C.

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THIS note describes a small furnace found useful in studying phase relations with the aid of microscopic observation between room temperature and about 350° C. The design problem is that of maintaining a temperature gradient of up to 100° C. per mm. in one direction (so that the sample can be at a high temperature with the microscope objective still at room temperature) but with substantially zero gradient in the plane perpendicular thereto, over an area of 2 sq. cm. Additional requirements are a means for rapid and accurate measurement of the temperature and a fairly low lag in varying the temperature.

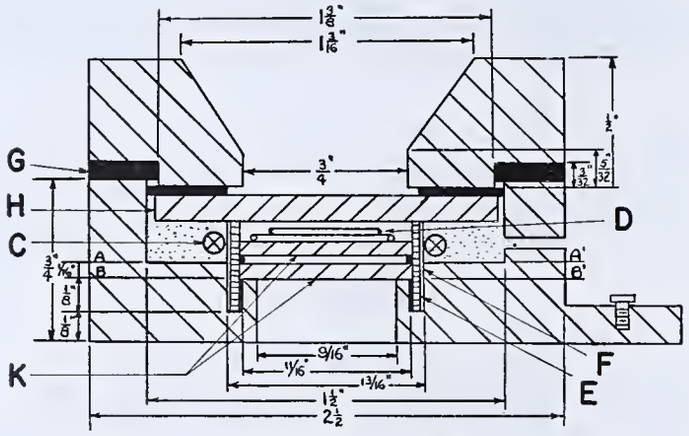

Figure 1. Cross Section of Microscope Hot Stage

C. Heating element
D. Capillary on wire coil
E. Brass ring
F. Mica strip
G. Asbestos disk
H. Pyrex disk
K. Glass disks

Commercially available heating stages are generally designed for biological applications and are not serviceable much above 50° C. Those few which can be heated to high temperatures are generally made of metal and therefore have a fairly high heat capacity which results in enormous temperature lag. The exterior of these stages becomes warm, with resultant inconvenience in manipulation. Metallic parts intended to distribute the heat uniformly usually undergo oxidation and deterioration with short use, and temperature inhomogeneity develops far greater than the usually claimed 1° above 200° C.

Custom-made hot stages for specific applications have been described (*1–4, 6, 11*). Wallace and Willard (*10*) devised a fairly simple, sturdy, and generally applicable hot stage not unlike the present design, which nevertheless suffered from several defects. The heated chamber was 2.5 cm. (1 inch) in depth and made of Alundum wound with Nichrome wire; this construction led to the development of severe vertical temperature inhomogeneity within the furnace proper. For use with a 10× objective the specimen had to be raised so close to the top of the stage that high temperatures were not attained readily, nor without the development of large horizontal temperature gradients.

The present furnace (Figures 1 and 2) is constructed of a lightweight porous fire brick (Johns-Manville magnesia brick). The bottom section consists of a block, 2.5 inches square and 0.75 inch high, from which the central portion has been drilled, leaving the terraces, *AA'* and *BB'*. A thin brass ring, 0.75 inch in diameter and $^5/_{16}$ inch high, fits into a slot, thus creating an annular space into which is inserted the heating element composed of a 1-foot length of No. 26 Nicrome resistance wire wound as a spiral. The heating element is wrapped around the brass ring fairly tightly and is separated from it by two strips of mica in order to prevent the development of hot spots. The brass ring, with winding, is then embedded in position with shredded asbestos paper. The leads to the heating element are brought out through holes drilled in the block, and secured to the binding posts as shown.

Two $^1/_{16}$-inch glass disks, separated by an air space, $^1/_{32}$-inch asbestos spacer, fit into the well created by the brass ring, leaving approximately $^1/_{16}$ inch from the top of the ring to the top glass disk. These glass disks serve to insulate the heated air chamber of the furnace from the condenser system of the microscope, as well as prevent too great a heat loss from the center of the main hot stage chamber. This is an effective technique, since the lower chamber is heated by the brass ring almost as effectively as is the upper chamber. The disks may be chipped from microscope slides—held under water while chipping to prevent cracking—or a circular glass cutter may be used.

The microscope objective is protected from the furnace by a Pyrex disk 0.125 inch thick, cut from a well-annealed plate. The latter condition is important if the furnace is to be used with a polarizing microscope, in order to prevent depolarization by the disk. The 0.125-inch disk is used rather than another dummy air space, as formed on the underside of the main hot stage chamber, because of its greater convenience when changing specimens. This disk is sufficiently thick to prevent large heat losses from the center of the heated air chamber—heat conduction from its periphery, where it makes contact with the asbestos insulation, probably accounts for much of the heat loss to the environment—but does not conduct so well as to serve as a source of heat for the air chamber. This was confirmed by using a pair of reversed thermocouples—one junction sealed to the glass disk and the other positioned in the center of the air space—and determining that the temperature of the underside of the disk was an average of 0.5° to 1.0° C. lower than the temperature of the air chamber.

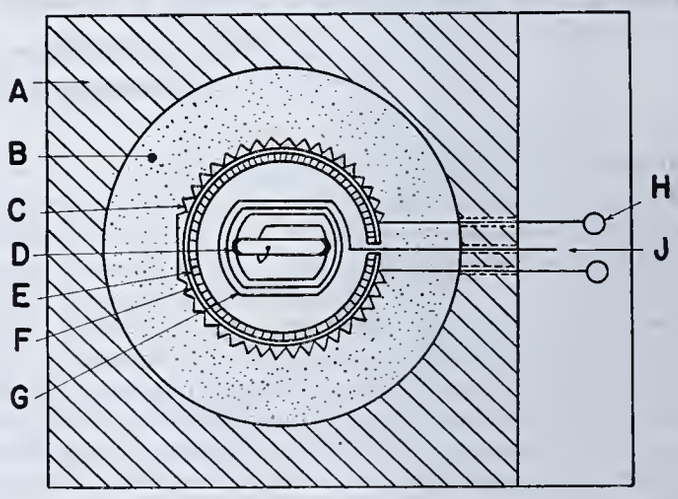

Figure 2. Section through Control Area of Hot Stage

A. Magnesia block
B. Asbestos paper
C. Heating element
D. Capillary
E. Brass ring
F. Mica strip
G. Thermocouple coil
H. Binding post
J. Thermocouple leads

A $^1/_{64}$-inch hole is drilled through the block and the brass ring to permit the entrance of a thermocouple for measuring the temperature within the ring. A thermocouple is preferred to a thermometer because of its much smaller heat capacity and its ability to measure a much wider range of temperature.

Copper-constantan thermocouple wire, No. 30 B. & S. gage, has been used successfully. Since the e.m.f. is 0.04 to 0.05 mv. per ° C., a Leeds & Northrup potentiometer and galvanometer, with a sensitivity of 0.015 microampere per mm., is satisfactory for measurements good to 0.3° C. If two or three loops of the thermocouple wire are left within the air space, conduction of heat along the wire is low enough so that standard calibration tables may be used. Otherwise, the calibration may run 2° to 3° C. lower than the reference tables at 300° C.

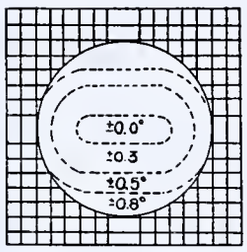

Figure 3. Temperature Difference between Center of Hot Stage and Other Parts

1 division = 1/16 inch. Determined at 250° C.

With this design, the air space within the brass ring can be maintained at temperatures up to 350° C. with the entire exterior of the block only faintly warm to the touch. Moreover, the heat capacity is sufficiently low so that a small change in heat input is reflected in a rapid adjustment of the temperature to its new value. The maximum rate of temperature rise compatible with maintenance of uniform temperature within the air space is 1.5° to 2.0° C. per minute. The temperature gradient under such circumstances is illustrated in Figure 3, which was determined by using two reversed thermocouple junctions and a galvanometer sensitive to 0.1° C.

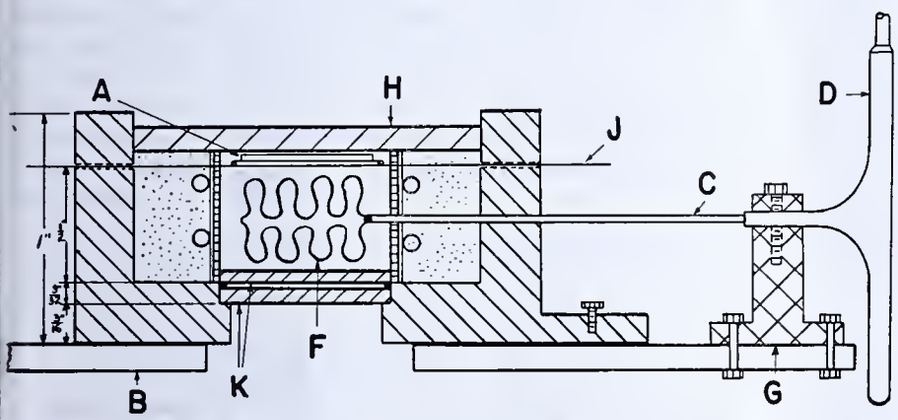

Figure 4. Cross Section of Modified Hot Stage

A. Microslide on wire coil
B. Microscope stage
C. Fan shaft
D. Air-driven rotor
E. Fan blade
G. Brass anchor block
H. Pyrex disk
J. Thermocouple lead
K. Glass disks

The temperature rise is controlled by means of a variac and a 12-ohm resistance, which is interposed in series between the furnace and the variac. The mean spontaneous rate of cooling with the heating element turned off is 20° C. per minute between 300° and 100° C., and 10° C. below 100° C.

The minimum distance from the microscope objective to the center of the heated space is approximately 4.2 mm., which with the usual design of microscope makes feasible the use of a 16-mm. (10×) objective, so that a magnification of the order of 100-power can be readily obtained. For intermittent observations the 0.125-inch Pyrex disk may be replaced by a 1/32-inch disk, permitting the use of a 20× objective. However, under such circumstances the heating rate should be below 0.7° to 1.0° C. per minute and the temperature should not exceed 200° to 250° C. in order to maintain the temperature homogeneity as indicated above.

The sample has to be mounted on a slide, cover slip, or other mount that can be contained entirely within the heated space. The field of view is adjusted entirely by moving the whole furnace about on the microscope stage. With a rotating stage, the furnace can be fastened to it, and moved with the adjustable spacers. The authors have been studying soap systems which must be protected from the air during heating and from vapor loss (*7, 8, 9*). It has proved convenient to contain such samples in sealed, flat capillaries about 12 to 15 mm. long, supported on a wire coil of the thermocouple wire, which in turn rests on the surface of the uppermost bottom glass cover of the furnace (see diagrams).

To test the accuracy of the hot stage, melting points were determined on the following:

Substance	Temperature, ° C. Observed	Reported
Tin	231.7 ± 0.3	231.9[a]
Urea	132.7 ± 0.3	132.6

[a] National Bureau of Standards sample.

In addition, phase changes in the system sodium nitrate–silver nitrate were determined to see if the points at the liquidus and solidus curves could be determined with accuracy. These results are:

Mole % Sodium Nitrate	Phase Change	Temperature, ° C. Observed	Reported (*5*)
0.00	Melting	208.8 ± 0.3	208.6
29.9	Melting	217.5 ± 0.3	217.5
	Freezing	235.2 ± 0.3	235.0
100.00	Melting	308.1 ± 0.3	308

In some cases, where the sample is spread over a more or less circular area, the temperature homogeneity may be insufficient for the most exacting work. To provide for such cases the authors have developed a modification of the furnace. The necessary changes in construction are indicated in Figure 4, and the temperature distribution obtained is shown in Figure 5.

In this modification the bottom section is 1 inch high. The brass ring is 9/16 inch high and is wrapped with two turns of coiled Nichrome wire instead of one, allowing for a heated space slightly greater than 0.5 inch in depth. Homogeneity is secured by the use of the stirrer blade, which is cut out from a piece of sheet copper, 9/16 by 5/8 inch. Eight blades are cut out as shown, and bent so as to circulate the air across the air space. The Pyrex disk should be cut to make a fairly tight fit in order to prevent leakage. The shaft, made of 1/32-inch Bessemer steel rod, is silver-soldered to the blade. The block and the brass ring are slotted to receive the shaft, so that the blade clears the top disk by at least 1/32 and preferably 1/16 inch. The shaft is driven by an air motor (Aero-Mix, Precision Scientific Co.) which is clamped by a brass block directly to the microscope stage, so that it is moved integrally with it. The specimen is again supported on a coil of the thermocouple wire, which is stiff enough to be suspended as shown.

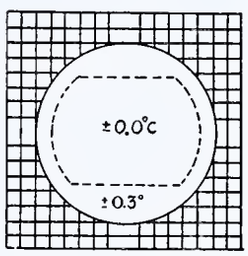

Figure 5. Temperature Distribution with Modified Hot Stage

1 division = 1/16 inch. Determined at 250° C.

ACKNOWLEDGMENT

The authors wish to express their appreciation to R. D. Vold for his interest during the development of this instrument.

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A Variable Pressure Manostat

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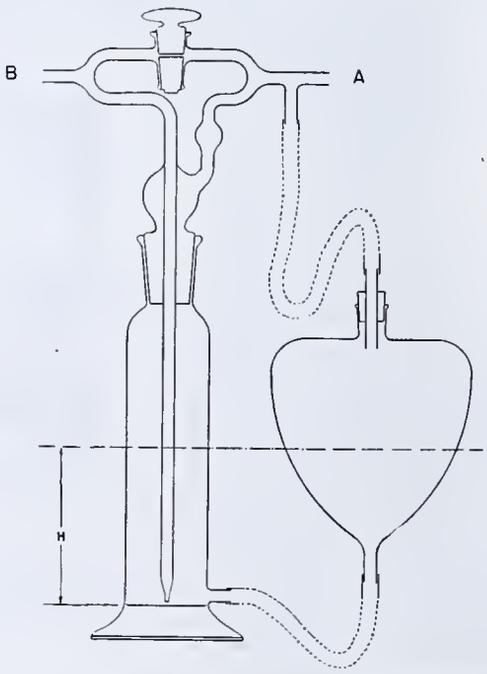
IT IS often convenient and sometimes necessary to reproduce or vary the pressure on a given system. The diagram shows an arrangement by which pressures may readily be set at any desired value or varied continuously, between limits determined by the efficiency of the pump and the height of the manostat, without removing the manostat from the vacuum line or interrupting the progress of the distillation.

Most vacuum distillations are conducted within the range from 1 to 250 mm. Any pressure within this range can easily be obtained by using a regulator of the Newman type (*1*) and inserting into the system a reservoir of mercury. Use of ethyl phthalate limits the range but makes possible more delicate setting. The height, H, of the liquid in the manostat can be changed manually during a distillation merely by adjustment of the reservoir (see diagram). A stopcock placed below the reservoir may be used to assure a constant head after setting. The entire assembly can be blown to order, but it is possible to make a very creditable model from a drying column.

Pressures above 250 mm. can be obtained by placing in series with the manostat described above two ordinary Newman regulators filled to the 250-mm. level. If the stopcocks are set so that both these regulators are by-passed, the range will be as stated above. With one tube cut in the range will be 250 to 500 mm.; with both, 500 to 750 mm.



A. To pump
B. To system

In the authors' opinion such an apparatus provides a more inexpensive and convenient method for checking boiling points at the pressures recorded in the literature than any heretofore described. The manostat has proved especially useful in the fractional distillation of mixtures when it is advantageous to distill the various components under successively lower pressures.

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Improved Adsorption Vessel

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IN THE study of metal-oxide catalysts such as used in the Fischer-Tropsch process by low-temperature adsorption isotherms, it is desirable to reduce the catalyst in the adsorption vessel and to determine the weight loss upon reduction. The adsorption vessel shown in Figure 1 has proved convenient in such studies. It is small and light enough to be weighed in an analytical balance.

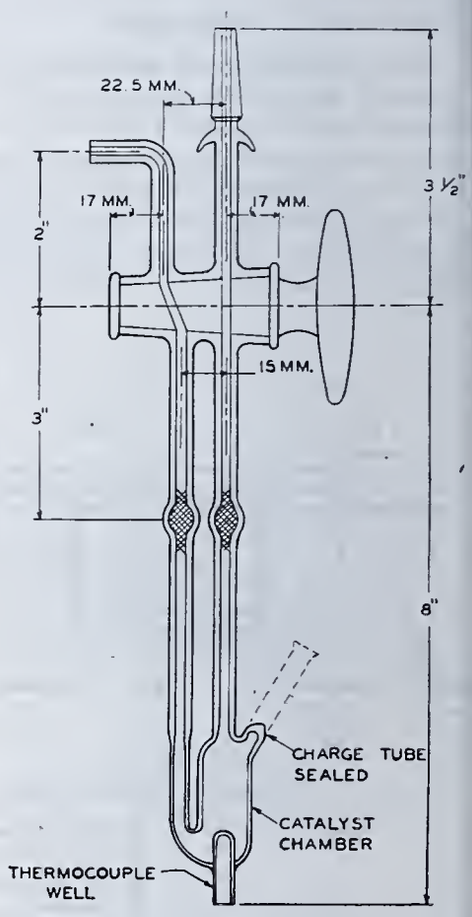

Figure 1

The absorption vessel consists of a catalyst chamber and a special four-way stopcock. The bores of the stopcock plug are arranged so that both are open in the position shown, while at an angle of 180° from this position only the right bore is open. The stopcock plug is of hollow blown construction, and the diameters of bores and capillary tubing are 2 mm. The catalyst chamber is made just large enough to accommodate the sample required, usually 1 to 2 cc. The catalyst chamber is provided with a tube for charging and a thermocouple well. The tubes leading to the stopcock contain loosely packed wads of glass wool to prevent the catalyst from blowing out of the chamber. The adsorption vessel is connected to the adsorption or reduction systems by a 10/30 capillary $\overline{\S}$ ground-glass joint.

With the four-way stopcock the sample can be connected to the system for adsorption or evacuation with one bore open, and with both bores open a stream of hydrogen can be passed over the catalysts. The sample of catalyst usually is weighed in the adsorption vessel before the charge tube is sealed. After sealing all weighings are performed with the vessel evacuated. Fresh samples are evacuated for an hour at 125° C. to remove water and other vapors that are held physically. The catalyst chamber is placed in a furnace in a horizontal position to avoid heating the stopcock.

Usually nitrogen adsorption isotherms at −195° C. were determined on the sample before and after reduction, and in some instances several isotherms were run during the course of reduction. With this adsorption vessel it also will be possible to study the effects of treatment of the catalyst with gases other than hydrogen, such as synthesis gas or carbon monoxide, on the surface area and pore structure, and to determine the weight loss of the adsorbent upon evacuation.

ACKNOWLEDGMENT

Acknowledgment is made to Martin Michael for preparation of the drawing. The special stopcock was made by Eck & Krebs.

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Determination of the Boiling Range of Chlorinated Hydrocarbons

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A study of the factors that affect precision in the determination of distillation temperatures of chlorinated hydrocarbons showed that thermometer calibration errors cause some variation. The effect of variations in atmospheric conditions, such as the ambient temperature and drafts, is appreciable. The rate of distillation is important in some cases. The most important single factor is superheating of vapor. Because, over limited ranges, distillation temperatures are linear functions of the composition, it is possible to determine the composition of most binary mixtures and some ternary mixtures by this method.

SEVERAL years ago this corporation initiated a study of the determination of boiling ranges of relatively pure commercial solvents such as carbon tetrachloride and trichloroethylene. A study of the effect of numerous impurities on the boiling range of pure solvents showed that, over limited ranges, distillation temperatures were linear functions of the composition. Thus, it is possible to determine the composition of most binary mixtures and some ternary mixtures very simply from distillation temperatures.

A study of the precision of the determination of distillation temperatures showed that it was possible to estimate relatively small concentrations of impurities from such data. However, the precision was far poorer than the precision with which a mercury-in-glass thermometer may be read. This led to a study of the factors which affect precision. Thermometer calibration errors caused some variation. A relatively simple piece of equipment was built for calibrating boiling range thermometers. The effect of variations in atmospheric conditions, such as the ambient temperature and drafts, was studied and was shown to be appreciable, at least under extreme conditions. The rate of distillation was shown to be important in the case of some solvents, but not others.

The most important single factor contributing to lack of precision is probably superheating of the vapor. While no satisfactory solution to this problem has been found, it is still under investigation and it is planned to make this the subject of a subsequent paper. It is believed that the present paper will prove of some interest, since methods of this type are in general use for testing the purity of fluids.

APPARATUS

The shield, condenser, distilling flask, and graduate are those specified in A.S.T.M. Designation D86-38 for the determination of the boiling range of gasoline. The following apparatus differs from that specified in the A.S.T.M. method:

HEATER. The heater sold under the trade name Ful-Kontrol is used. The upper refractory is 1.9 cm. (0.75 inch) thick and has a 2.5-cm. (1-inch) hole in the center. The refractory is used plane side up.

THERMOMETER. Precision grade, length 40 cm. (16 inches), scale 23.8 cm. (9.5 inches), immersion 10 cm. (4 inches) including bulb 1.9 cm. (0.75 inch) in length. Scale starts 2.5 cm. (1 inch) above line of immersion, expansion chamber above bulb and above column, distance from bottom of bulb to top of lower expansion chamber not over 3.8 cm. (1.5 inches), scale 40° C. in 0.1° C. divisions. Thermometers conforming to these specifications and covering any desired range may be purchased from Taylor Instrument Companies on special order. The thermometers are calibrated at the boiling point of each fluid with which they are used, according to the procedure described below.

THERMOMETER READER. Central Scientific Company Catalog 19,520 or its equivalent.

PROCEDURE

The heater is clamped into the position in which it is to be used during the distillation. A tight connection is made by means of a cork between the vapor tube of the flask and the condenser tube. The flask is adjusted so that its outlet extends into the condenser tube 2.5 to 5 cm. (1 to 2 inches). The thermometer, provided with a cork, is tightly fitted into the flask, so that it is in the middle of the neck of the flask and the top of the expansion chamber is level with the inside of the bottom of the outlet tube at its junction with the neck of the flask. The immersion mark should be close to the bottom of the cork. Water at approximately room temperature is allowed to circulate through the bath.

The heater is turned on long enough before starting the distillation to maintain a constant rate of distillation. The heater must be turned on at the voltage at which it is to be used for at least an hour, or for a shorter time at a higher voltage, before starting the distillation. The voltage required to give a constant rate of distillation of 5 to 6 ml. per minute is determined empirically for each fluid.

A 100-ml. sample, which is approximately at room temperature, is measured in a dry, 100-ml. graduated cylinder and transferred to the dry distillation flask. The thermometer is placed in the charged flask and the flask is fitted to the condenser. The flask must be carefully fitted onto the hole in the refractory of the heater. The graduated cylinder is placed without drying under the outlet of the condenser.

The temperature is read and the time recorded when the first drop falls into the graduated receiver. The receiver is then moved so that the tip of the condenser touches the side of the receiver. The temperature is read when the volume in the graduate reaches 5 ml. and 95 ml., and when the distilling flask just goes dry. All temperature readings are made by means of the thermometer reader and are recorded to the nearest 0.01° C. The time is recorded at the dry point. The time between the first drop and the dry point should be between 17 and 20 minutes.

The barometer and the temperature alongside are read during the course of the distillation. The pressure is corrected for brass scale as follows:

$$P_0 = P_t - (t \times P_t \times 0.000160)$$

where P_0 is the barometric pressure in millimeters of mercury at 0° C. and P_t is the pressure at temperature t. The distillation temperatures are corrected to 760-mm. pressure as follows:

$$T_{760} = T_{obs.} + dT/dP\ (760 - P_0)$$

where T_{760} and $T_{obs.}$ are the corrected and observed distillation temperatures, respectively.

These formulas may be simplified in a given laboratory in the

Table I. Precision of Boiling Range of Carbon Tetrachloride under Best Conditions

Test No.	Temperature, ° C. First drop	5 ml.	95 ml.	Dry	Range
1	76.58	76.71	76.80	76.89	0.31
2	76.58	76.73	76.80	76.88	0.30
3	76.56	76.72	76.80	76.90	0.34
4	76.58	76.73	76.80	76.90	0.32
5	76.56	76.71	76.79	76.84	0.28
6	76.58	76.72	76.80	76.87	0.29
7	76.53	76.69	76.78	76.87	0.34
8	76.59	76.72	76.79	76.86	0.27
9	76.57	76.72	76.79	76.87	0.30
10	76.56	76.72	76.79	76.83	0.27
Av.	76.57	76.72	76.80	76.87	0.30
σ_1 of group	±0.016	±0.011	±0.008	±0.022	±0.024
LU_1 of method	±0.054	±0.036	±0.024	±0.072	±0.081

interest of expediting routine work. The following simplified formulas are used in this laboratory:

$$P_0 = P_t - (t \times 0.122)$$

$$T_{760} = T_{obs.} + f\,(760 - P_0)$$

The factor f is 0.044° C. per millimeter for carbon tetrachloride. Finally, the thermometer calibration correction is added.

MATERIALS

With few exceptions the materials used in this work were the normal commercial products. It was assumed that a product of sufficient purity was being used when the specific gravity and the boiling point agreed closely with the accepted value found in the literature. In case the normal commercial product was not sufficiently pure, a selected product was obtained or the material was purified by rectification in the laboratory.

EXPERIMENTAL

Precision. The precision of the boiling range method as applied to a number of fluids was determined according to the procedure outlined by Moran (*3*). Data for a typical solvent, commercial carbon tetrachloride, are shown in Tables I and II. As was pointed out by Moran, the high ratios of LU_2:LU_1 (limit of uncertainty under routine conditions to limit of uncertainty under the best conditions) indicates a method having considerable personal and seasonal variations. Nevertheless, these data are useful in estimating the precision within which impurities in carbon tetrachloride and other solvents may be determined from boiling range data.

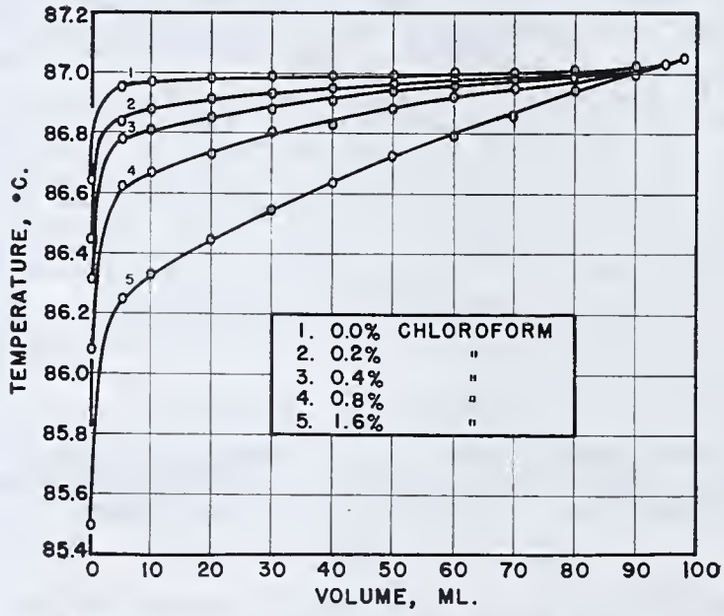

Figure 1. Effect of Chloroform on Boiling Range of Trichloroethylene

Effect of Impurities. The boiling ranges of trichloroethylene to which known amounts of chloroform have been added are shown in Figure 1. These data are typical of those obtained for binary mixtures. By plotting the temperature of the 5-ml. point against concentration it can be shown that the chloroform concentration over the range 0 to 16 volume per cent is given by:

$$c = 2.39\,(86.93 - t)$$

where c is the concentration in volume per cent and t is the temperature in ° C. The LU_1 of the 5-ml. temperature for trichloroethylene is ±0.036° C. and the LU_2 ±0.11° C. Thus, under the best conditions, it would be possible to determine the concentration of chloroform in trichloroethylene within ±0.09%, while under routine conditions the concentration of this impurity could be determined within ±0.26%.

Data for the calculation of a number of impurities in different solvents are summarized in Table III. The carbon tetrachloride which was used in obtaining these data was the commercial product, since the boiling range data showed this product to be pure within the precision of the method. The trichloroethylene, perchloroethylene (tetrachloroethylene), and acetylene tetrachloride (1,1,2,2-tetrachloroethane) were rectified to obtain materials having narrow boiling ranges. The purities of the trichloroethylene and perchloroethylene were comparable to that of the carbon tetrachloride. The acetylene tetrachloride was somewhat less pure, the boiling range from the 5-ml. point to the dry point being 0.8° C.

The concentrations of all low-boiling impurities were calculated from the temperature at the 5-ml. point, while the concentrations of high-boiling impurities were calculated from the temperature at the dry point. Low-boiling impurities have a somewhat greater effect on the first-drop temperature than on the temperature at the 5-ml. point, but the precision of the latter temperature is greater than that of the former, and in general more than compensates for the lower slope of the temperature-composition curve. For example, the LU_2 of the first-drop temperature of trichloroethylene is three times that of the LU_2 of the 5-ml. point. In determining chloroform in trichloroethylene, the slope of the temperature-composition curve, expressed in degrees per per cent, is twice as large for the first drop as for the 5-ml. point. In the determination of ethylene dichloride (1,2-dichloroethane) in trichloroethylene it is 1.4 times as great. It is seen that the increase in slope does not compensate for the reduced precision. A further indication of the relative precision is a greater scatter-

Table II. Precision of Boiling Range of Carbon Tetrachloride under Routine Conditions

Date	Analyst	Temperature, ° C. First drop	5 ml.	95 ml.	Dry	Range
Jan.	1	76.60	76.72	76.83	76.91	0.31
	1	76.56	76.78	76.84	76.96	0.40
Feb.	2	76.64	76.79	76.88	76.99	0.35
	2	76.64	76.81	76.90	77.00	0.36
March	1	76.63	76.75	76.83	76.90	0.27
	1	76.63	76.75	76.87	76.94	0.31
April	3	76.57	76.68	76.79	76.86	0.29
	3	76.56	76.68	76.78	76.86	0.30
May	1	76.52	76.79	76.89	77.01	0.49
	1	76.43	76.78	76.89	76.99	0.56
	1	76.58	76.76	76.83	76.90	0.32
June	3	76.61	76.78	76.85	76.90	0.29
	3	76.61	76.79	76.85	76.90	0.30
July	1	76.54	76.81	76.87	76.94	0.40
	1	76.55	76.80	76.87	76.92	0.37
Aug.	4	76.60	76.78	76.85	76.92	0.32
	4	76.55	76.77	76.82	76.90	0.35
Sept.	4	76.59	76.77	76.83	76.88	0.29
	4	76.58	76.76	76.82	76.89	0.31
Oct.	5	76.55	76.71	76.77	76.83	0.28
	5	76.64	76.76	76.86	76.88	0.24
Nov.	5	76.60	76.73	76.80	76.86	0.26
	5	76.62	76.77	76.82	76.88	0.26
Dec.	6	76.50	76.76	76.85	76.92	0.42
	6	76.60	76.75	76.81	76.88	0.28
	Av.	76.58	76.76	76.84	76.91	0.33
	σ_2 of group	±0.05	±0.035	±0.035	±0.05	±0.075
	LU_2 of group	±0.15	±0.11	±0.11	±0.15	±0.23
	Ratio LU_2:LU_1	3.0	2.8	5.5	2.1	2.9

Table III. Calculation of Impurities in Solvents from Boiling Range Data

Substance	Impurity	Equation	Range[a] %	LU_1 ° C.	LU_2 ° C.
CCl_4	(1,2) $C_2H_4Cl_2$	$c = 8(76.72 - t)$	0 to 2	±0.036	±0.11
CCl_4	C_2HCl_3	$c = 2.56(t - 76.87)$	0 to 1	±0.072	±0.15
CCl_4	C_2Cl_4	$c = 0.077(t - 76.87)$	0 to 1.6	±0.072	±0.15
CCl_4	C_2Cl_6	$c = 0.031(t - 76.87)$	0 to 0.02	±0.072	±0.15
C_2HCl_3	$CHCl_3$	$c = 2.39(86.93 - t)$	0 to 1.6	±0.036	±0.11
C_2HCl_3	(1,2) $C_2H_4Cl_2$	$c = 5.71(86.93 - t)$	0 to 4	±0.036	±0.11
C_2HCl_3	C_2Cl_4	$c = 0.187(t - 87.05)$	0 to 1	±0.087	±0.17
C_2Cl_4	CCl_4	$c = 1.26(121.08 - t)$	0 to 1	±0.02	...
C_2Cl_4	C_2HCl_3	$c = 1.70(121.08 - t)$	0 to 4	±0.02	...
C_2Cl_4	C_2HCl_5	$c = 0.140(t - 121.34)$	0 to 3	±0.05	...
C_2Cl_4	C_2Cl_6	$c = 0.069(t - 121.34)$	0 to 2	±0.05	...
(1,1,2,2) $C_2H_2Cl_4$	(1,2) $C_2H_4Cl_2$	$c = 0.71(145.5 - t)$	0 to 2	...	...
(1,1,2,2) $C_2H_2Cl_4$	C_2HCl_5	$c = 1.33(t - 146.3)$	0 to 8	...	...
(1,1,2,2) $C_2H_2Cl_4$	C_2Cl_6	$c = 0.22(t - 146.3)$	0 to 4	...	...

[a] Liquid impurities in volume %; solids in weight %.

ing of the points when the temperature-composition curve is plotted for the first drop than when it is obtained for the 5-ml. point. The precision of the measurement of the dry-point temperature is normally slightly poorer than that of the 95-ml. temperature, but this slight decrease in precision only partially offsets the greater slope in the temperature-composition curve.

The ranges over which linear equations apply are shown in Table III. For the most part, linear equations applied over the entire range tested and presumably over even wider ranges.

In general, one impurity, if present in significant amounts, affects the entire boiling range. Thus, the boiling range method is generally applicable only for the analysis of binary mixtures. For example, the presence of chloroform, ethylene dichloride, or acetylene tetrachloride in carbon tetrachloride prevents the quantitative determination of any of the others. Chloroform or ethylene dichloride may, on the other hand, be present in trichloroethylene without interfering with the determination of high-boiling impurities. Ethylene dichloride does not interfere with the recovery of either pentachloroethane or hexachloroethane from acetylene tetrachloride, nor do either of the latter interfere with the former over the ranges given in Table III.

In general, commercial products contain both low-boiling and high-boiling impurities and the identity of the impurities may not be known. While the boiling range cannot be used for either the qualitative or the quantitative analysis of such fluids, it is useful in indicating the presence of both low-boiling and high-boiling impurities. Further, an indication of the difference between the boiling point of the main component and that of the impurity can be obtained from the rate of change of temperature with respect to the volume. Possibly the most useful application of the method is in the study of intermediates. It may be applied to supplement specific gravity and refractive index data for the analysis of complex mixtures. As is shown in Table II, very small concentrations of impurities can be detected from boiling-range data in case of compounds which have a substantial difference in boiling points and even when the difference in boiling points is relatively small the method is reasonably sensitive. The smallest difference in boiling points is that between trichloroethylene and ethylene dichloride (4° C.). The data show that ethylene dichloride may be detected in trichloroethylene in concentrations as small as 0.5%. Where a wide difference exists between the boiling points, as in the case of hexachloroethane in carbon tetrachloride, concentrations as small as 0.005% may be detected.

CALIBRATION OF THERMOMETERS. A coaxial constant-temperature bath similar to that used by the National Bureau of Standards (*1*) was constructed for the calibration of thermometers. The bath consisted of a 25 × 45 cm. (10 × 18 inch) Pyrex jar. The sides were covered with a 3.8-cm. (1.5-inch) layer of Fiberglas insulation and the bottom with a 5-cm. (2-inch) layer of magnesia. The cover consisted of two circles of 0.6-cm. (0.25-inch) Transite bolted together, one circle fitting inside and one outside the glass jar. The cover was split for convenience and slots were cut in it for insertion of the stirrer, heater, and thermometers. The inside cylinder was a 7.5-cm. (3-inch) metal tube. The heat input was controlled by means of a Variac. A temperature of 85° C. could be maintained with a heat input of about 80 watts.

The standard thermometers were purchased from Taylor Instrument Companies, Rochester, N. Y., under the following specifications: precision grade, length 45 cm. (18 inches), total immersion, with ice point, expansion chamber at top of column, length of scale 30 cm. (12 inches), range 40° C. in 0.1° C. divisions. The manufacturer supplied a laboratory test certificate with each thermometer at the ice point and each 5° C. The ice points were checked prior to use. These thermometers were subsequently calibrated by the Bureau of Standards.

The following procedure was used for the calibration: The bath was adjusted to the temperature at which the calibrations were to be made. The standard thermometers were placed in the bath with 2.5° C. emergent stem measured to the center of the cover. The distillation thermometers (several were calibrated simultaneously) were adjusted to their immersion marks. The heat input to the bath was adjusted by means of the Variac, so that the temperature rose 0.1° to 0.2° C. during the time required to make a set of readings, which was about 10 minutes. The thermometers were then read in rotation beginning with the standard. Two laboratory-grade thermometers, which were used to measure the stem temperatures of the standard and distillation thermometers, respectively, were then read, after which the series was read in the reverse order. This cycle was repeated five times, making a total of ten readings for each thermometer. The average of the ten readings was calculated for each thermometer. This average for the standard was corrected for emergent stem (about 0.03° C.) and for the calibration correction given by the manufacturer. The difference between this temperature and the average temperature calculated for the distillation thermometer is the calibration correction for the latter.

Thermometers were calibrated at the boiling points of the fluids with which they were to be used. The maximum calibration error observed was 0.17° C. The precisions of the calibrations were checked under actual working conditions by making distillation ranges. The distillation ranges for carbon tetrachloride, using calibrated thermometers, are shown in Table IV. Slight differences are indicated between the several calibrated thermometers. These data were taken over a period of several weeks by one person. The precision of the entire series falls between the LU_1 and LU_2 values given in Tables I and II. A precision of this order of magnitude is to be expected under these conditions. It is therefore improbable that there is any significant difference between the distillation temperatures given by the calibrated thermometers.

Variations in the emergent stem temperature will cause significant errors in apparent distillation temperatures. The temperature of the midpoint of the emergent stem during the calibration of thermometers at the boiling point of trichloroethylene was approximately 10° C. above that of the room. The temperature of the midpoint of the emergent stem during distillation varied from 10° to 20° C. above that of the room. Seasonal

Table IV. Distillation Ranges of Carbon Tetrachloride Using Calibrated Thermometers

Thermometer No.	Calibration Correction, ° C.	Temperature, ° C. First drop	5 ml.	95 ml.	Dry
1,977,258	−0.09	76.42	76.61	76.70	76.77
	−0.09	76.41	76.61	76.70	76.73
2,172,778	−0.17	76.41	76.62	76.70	76.76
	−0.17	76.41	76.61	76.67	76.74
1,977,259	−0.09	76.39	76.61	76.69	76.77
	−0.09	76.41	76.60	76.69	76.78
2,284,595	−0.13	76.43	76.65	76.73	76.76
	−0.13	76.44	76.65	76.72	76.77
	−0.13	76.47	76.66	76.72	76.77
5,677	−0.05	76.44	76.65	76.73	76.79
	−0.05	76.45	76.65	76.72	76.80
	−0.05	76.36	76.66	76.74	76.83
5,678	−0.02	76.43	76.65	76.71	76.79
	−0.02	76.40	76.64	76.72	76.81
5,679	−0.08	76.43	76.68	76.74	76.83
	−0.08	76.43	76.66	76.75	76.83
5,680	−0.05	76.39	76.67	76.74	76.82
	−0.05	76.40	76.67	76.74	76.83

variations in room temperature of 15° C. are not uncommon. Thus, the temperature of the emergent stem during use may differ by 25° C. from that during calibration. The emergent stem of the particular thermometer which was used for this work (range 70° to 110°C.) measured about 20° C. during the distillation of trichloroethylene. Thus, errors up to 0.08° C. may result from this cause. If the top of the scale were used, this error would be twice this value. For the highest precision both the calibration and the distillation temperature must be corrected to the same emergent stem temperature. Since, however, this correction is subject to considerable uncertainty, the most precise results would be obtained by using a thermometer with a shorter range, say 10° C.

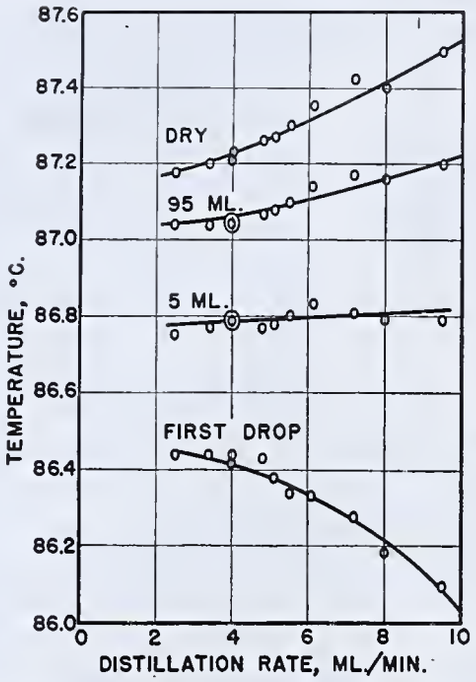

Figure 2. Effect of Rate on Distillation Temperature of Trichloroethane

Effect of Conditions. From Figure 2 it is seen that the rate of distillation has a marked effect on the distillation temperatures of trichloroethylene. It was found that the rate has a significant but less pronounced effect on the distillation temperatures of perchloroethylene, but it has no detectable effect on the distillation temperatures of carbon tetrachloride. Thus it appears that the rate has a marked effect on some fluids and none whatever on others. In general it is desirable to maintain the rate as nearly constant as possible. It is considered practical to maintain the rate constant within a range of about 1 ml. per minute. A rate of 5 to 6 ml. per minute was chosen in order to complete the analysis as rapidly as possible. To ensure that this rate is maintained, it is necessary that the time of the first drop and the dry point be recorded. No attempt is made to control the time between the application of heat and the first drop, since this is automatically controlled by the heat input required to maintain the desired rate.

The shield which is specified in the A.S.T.M. method for the distillation of gasoline was normally used during this work. The temperature inside the shield was substantially above that of the room, owing to heat radiated from the distillation heater. Calculation showed that the efficiency of the heater was only about 8%, the remaining 92% of the heat serving to raise the temperature of the surrounding atmosphere.

To exaggerate this effect a cover of asbestos paper was provided for the shield. Thus, the heater and flask were completely surrounded by the shield. The distillation thermometer protruded through a hole in the cover. An auxiliary thermometer to measure the temperature of the space inside the shield also extended through the cover. The bulb of this thermometer was about 10 cm. (4 inches) from the heater. This thermometer registered a temperature of 60° to 70° C. during the time the distillation was in progress. This additional shielding raised the distillation temperature of trichloroethylene at the 95-ml. and dry points by 4° and 8° C., respectively.

In an effort to reduce this effect, the preceding experiment was repeated except that the neck of the distillation flask was insulated by wrapping it with asbestos wicking. This relatively inefficient method of insulation reduced the effect on the temperature at 95-ml. point and the dry point to 2° and 5° C., respectively.

The effect of drafts was greatly exaggerated by removing the shield and directing a fan on the distillation flask. This procedure reduced the rate of distillation to 2 ml. per minute in spite of the fact that the heat input was more than doubled. Except for the high dry point, the distillation temperatures were not affected. This experiment was repeated except that the insulated flask described above was used. A normal boiling range was obtained under these conditions.

From these data it appears extremely doubtful whether the drafts which are normally present in the laboratory will have any significant effect on the boiling range of relatively pure liquids such as those tested in this work, even though the A.S.T.M. shield were omitted. Superheating of the vapors by the radiant energy lost from the heater occurs to a substantial degree and is magnified by any device which encloses the distillation flask within the same space occupied by the heater. While it cannot be definitely concluded that the shield is harmful, it appears extremely doubtful whether it serves any useful purpose. It appears probable that, if the elimination of the effect of atmospheric conditions is necessary, it should be done by insulating the flask and not by enclosing the flask and heater within a shield.

The author recognizes that this and perhaps other conclusions drawn from this work are contrary to commonly accepted beliefs and are apt to arouse controversy. Such conclusions cannot be considered as final, but if they serve to stimulate further investigation their purpose will have been served.

Since the most serious error is caused by the very low efficiency of the heater, the most promising method for improving the procedure would involve the design of a more efficient heater. Some work has been done along this line but a satisfactory solution has not been obtained to date. Formed-in-place heaters similar to those described by Krantz and Hufferd (*2*) were built to fit a 100-ml. distillation flask. Heaters of this design 6.25 cm. (1.5 inches) in diameter appear to have entirely eliminated superheating. Heaters of larger diameter cause superheating. That superheating is eliminated is shown by the following facts:

Using formed-in-place heaters, the rate has no effect on the distillation temperatures of trichloroethylene and the range of distillation temperatures from the first drop to the dry point is less than that which was obtained at the lowest rate at which the Ful-Kontrol heater was used. The Ful-Kontrol heater causes superheating of methylene chloride of as much as 10° C. near the end of the distillation, but there was no indication of superheating of methylene chloride when using a 3.75-cm. (1.5-inch) formed-in-place heater. While this type of heater appears to eliminate superheating entirely, it is difficult to construct and has a short life in the small size required for this work. Other types of heaters are being tested, but none has been found to date which is considered entirely satisfactory. This work is continuing and it is anticipated that a heater will eventually be found which is generally satisfactory. It is planned to make this the subject of a subsequent paper.

ACKNOWLEDGMENT

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Amperometric Titration of Mercaptans with Silver Nitrate

Using the Rotating Platinum Electrode

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THE rotating platinum wire electrode has been introduced as an indicator electrode in amperometric titrations (*2*) by Laitinen and Kolthoff (*3*). The performance of an amperometric titration with the rotating platinum electrode as indicator electrode becomes especially simple, when no e.m.f. needs to be applied to the cell consisting of the indicator electrode and the reference electrode.

The rotating electrode is placed in the solution to be titrated, electrolytic connection is made with the reference electrode, and the current which flows through the cell during the titration is read on a microammeter. The diffusion current of the substance titrated or of the reagent is measured at the potential of the rotating electrode. For example, in the titration of a mercaptan with silver nitrate using the saturated calomel electrode as a reference electrode, the current is zero until the end point. After the end point, when there is an excess of silver in the solution, the diffusion current of silver is measured with the microammeter, this diffusion current being proportional to the concentration of silver ions. When the current readings during the titration are plotted against the volume of reagent added, two straight lines are obtained, which intersect at the end point (see Figure 2). In the example under consideration, the current before the end point is practically zero and is equal to the residual current of the medium.

If the titration is carried out in ammoniacal medium as in the titration of mercaptans with silver in the presence of chloride, the potential of the saturated calomel electrode is not negative enough to yield the diffusion current of the amino-silver ions. Therefore, another reference electrode, which is more negative than the saturated calomel electrode, but not sufficiently negative to give cathodic currents of oxygen, has been used.

In the present paper the amperometric titration of very small amounts of primary, secondary, and tertiary mercaptans with silver nitrate is described. In the absence of chloride, this titration can be carried out in acid or neutral medium with the saturated calomel electrode as reference electrode. Only the procedure in ammoniacal medium is presented here, since much chloride and little bromide do not interfere in the presence of much ammonia. The titration in the presence of ammonia is as simple as that in neutral or acid medium.

The reaction equation is:

$$Ag^+ + RSH \longrightarrow RSAg + H^+$$

or

$$Ag(NH_3)_2^+ + RSH \longrightarrow RSAg + NH_4^+ + NH_3$$

RSH denotes a mercaptan.

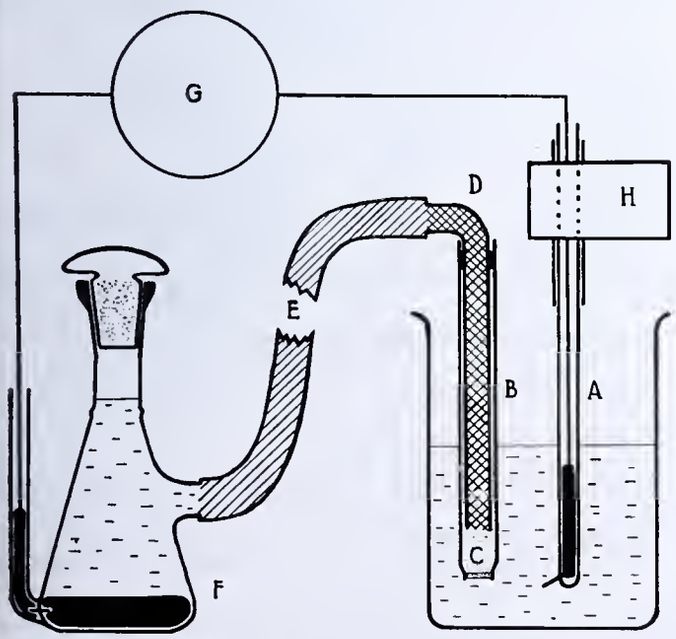

Figure 1. Apparatus for Amperometric Titration

APPARATUS AND MATERIALS

The apparatus and the circuit are shown in Figure 1.

A rotating platinum wire electrode, *A*, about 6 to 8 mm. long and 0.5 mm. in diameter, sealed in 6-mm. soft glass tubing, serves as indicator electrode. Since the titrations can be completed within a short time, an ordinary motor can be used to rotate the electrode. A synchronous motor is not necessary in titration work. For amperometric titration a simple rotating electrode can be made by replacing the shaft of an ordinary cone drive motor, *H*, with a short length of brass tubing (developed in this laboratory by D. G. Weiblen). The 6-mm. glass tubing with the electrode is fastened inside the brass tubing. Electrical contact is then readily made by dipping a wire in the mercury inside the glass tubing.

A reference electrode, *F*, is used which has a potential of −0.23 volt against the saturated calomel electrode. The electrolyte solution for the reference half-cell is prepared by dissolving 4.2 grams of potassium iodide and 1.3 grams of mercuric iodide in 100 ml. of saturated potassium chloride solution. A layer of mercury serves as the electrode.

Electrical connection between the reference and indicator electrodes is made by means of a salt bridge, *E*, consisting of about 60 cm. (2 feet) of 6-mm. (inside diameter) soft rubber tubing filled with saturated potassium chloride solution (*1*). The rubber tubing is connected with a short length of glass tubing, *D*, filled with a gel of 3% agar and 30% potassium chloride. At the end of the glass tube, a coarsely sintered glass disk may be inserted, if desired. For further protection of the solution from contamination with iodide, the glass tube, *B*, having an agar or a fine sintered-glass plug at its end, may be interposed. The electrolyte solution, *C*, inside *B* can be easily rinsed out and replaced with fresh electrolyte whenever it has been in use long enough to become contaminated with iodide. It is essential that all sources of high resistance, such as air bubbles, be eliminated from the salt bridge.

To complete the circuit the two half-cells are short-circuited through a microammeter, *G*. A Weston Electrical Instrument Corporation, Newark, microammeter (Model 430) has been used in the present work. Instead of the microammeter, other current-indicating devices may be used, such as a pointer galvanometer with sensitivity of 0.25 microampere per division on the attached scale.

The mercaptans tested were analyzed for mercaptan sulfur content by the potentiometric method of Tamele and Ryland (*5*). Sources of the various mercaptans were:

n-Dodecyl	Organic Chemical Division, University of Minnesota
Commercial primary C_{12}	U. S. Rubber Co.
Commercial tertiary C_{12}	Sharples Chemicals, Inc.
Cyclopentyl	C. S. Marvel, University of Illinois

PROCEDURE

In a 250-ml. beaker dilute a sample of mercaptan containing about 5 mg. of mercaptan sulfur to 100 ml. with 95% ethanol. Make the solution about 0.25 *M* in ammonia and 0.01 to 0.1 *M* with some noninterfering electrolyte such as ammonium nitrate. Immerse the end of the salt bridge and the rotating platinum electrode in this solution. Titrate with aqueous 0.005 *M* silver nitrate.

Make 2 or 3 readings of the microammeter before the end point. As long as the silver nitrate is not in excess the current is very small or zero. After the end point the deflection of the ammeter corresponds to the diffusion current of the excess of silver. When

Table I. Amperometric Titrations of Mercaptans

Mercaptan Used	Method of Taking Sample	Mercaptan Sulfur Present	Mercaptan Sulfur Found
		Mg.	*Mg.*
n-Dodecyl	Weighing	5.04	5.075
		5.48	5.490
	Dilution of standard solution	1.942	1.945, 1.943
		7.77	7.77, 7.80
		1.93	1.89, 1.93
Commercial primary C_{12}	Weighing	5.26	5.249
		5.01	5.006
Commercial tertiary C_{12}	Weighing	6.47	6.456
		6.94	6.944
Cyclopentyl	Dilution of standard solution	3.047	3.051

the ammeter indicates the end point is passed add two or three small increments of silver nitrate; read the current after each addition. If the electrode becomes sluggish, clean, by wiping with a piece of cloth or between two fingers. Plot the readings of the microammeter against the volume of silver nitrate added. Draw two straight lines (*A* and *B*, Figure 2); the point of intersection, *C*, corresponds to the end point.

A titration carried out by an experienced operator requires no more than 2 minutes.

RESULTS OF TITRATION

Table I shows the results obtained with various mercaptans. The figures of mercaptan sulfur present are based upon the results of potentiometric titrations of large samples. With 5 mg. or more of mercaptan sulfur the amperometric titrations were reproducible within 0.2%.

The current readings obtained during the titration of 1.942 mg. of *n*-dodecyl mercaptan sulfur are shown in Figure 2. The method has been applied to a large number of other mercaptans with satisfactory end-point determinations in all cases. Among mercaptans that have been titrated are: primary C_4, C_6, C_8, C_{10}, C_{14}, C_{16}, and C_{18}; secondary C_8 and C_{12}; tertiary C_4, C_6, C_7, C_8, C_{10}, C_{14}, and C_{16} as well as thiols with other functional groups.

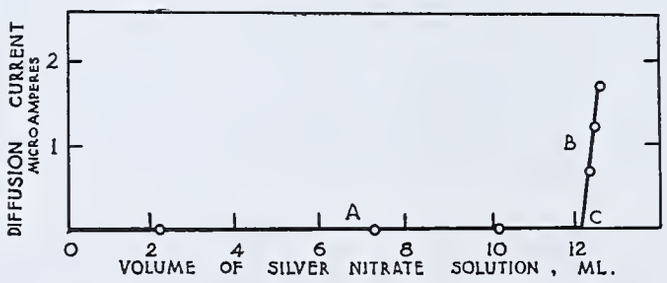

Figure 2. Amperometric Titration of 1.942 Mg. of Mercaptan Sulfur with 0.00495 *N* Silver Nitrate

Note. An indicator electrode may become insensitive or erratic after long use or when titrating large amounts of mercaptan. As suggested by May (*4*) full sensitivity can be restored by wiping the electrode with a piece of cloth or even between two fingers. In the most troublesome cases the following procedure is recommended in determining the end point.

After the addition of the first excess of silver nitrate as indicated by the first slight deflection of the ammeter stop the rotating electrode, wipe, and start again. Read the current immediately; add small increment of silver nitrate. Stop the rotating electrode, wipe, and continue as before. Read the current immediately after starting the electrode. Repeat the above operations 3 to 4 times. The end point is obtained from the readings in the manner previously described.

Thorough cleaning of the electrode with concentrated nitric acid is usually necessary only after it has been used for several hundred titrations. When a new or freshly cleaned electrode is placed in the ammoniacal mercaptan solution and the cell is short-circuited, a large current of 20 to 30 microamperes may be observed. This current decreases rapidly and is practically zero after waiting for 5 to 10 minutes.

Infrequently the glass in the region of the glass-to-platinu seal may become very slightly cracked. In such case a new ele trode must be prepared.

In some cases much suspended material in solution interfer mechanically with the current readings. The use of an electro of the design shown in Figure 3 eliminates interferences of th nature.

INTERFERENCES

Cyanide in ammoniacal medium forms a stable complex wit silver ions and interferes in the titration. Other ions, like iodic and sulfide, which form insoluble silver salts in ammoniac medium, also interfere. It is a simple matter to separate th mercaptan from interfering ions by shaking the mercaptan out i ether or some other suitable organic solvent.

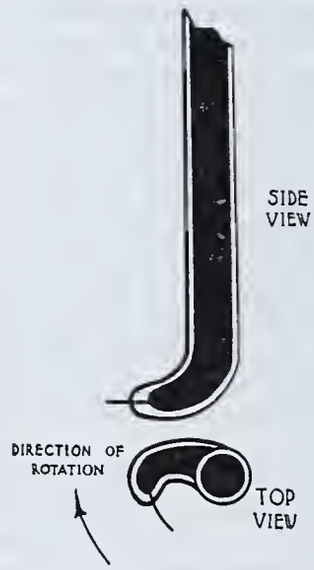

Figure 3. Rotating Platinum Electrode

Large amounts of chlorid and small amounts of bromi do not interfere in the pr cedure (Table II).

SUMMARY

A simple, rapid, and accurat amperometric titration metho for the routine determinatio of primary, secondary, an tertiary mercaptans with silve nitrate is described, using th rotating platinum wire elec trode as indicator electrode The apparatus required i simple and is available in mos laboratories.

Amounts of mercaptan sulfu as small as 0.2 mg. in 100 m of ethanol can be determine with an accuracy of 1 or 2% Amounts greater than 2 mg per 100 ml. can be determine with an accuracy and precision of at least 0.3%. The time re quired for performance of the entire titration need not be greate than 2 minutes. In ammonical medium, large amounts of chlo ride and small amounts of bromide do not interfere. Larg amounts of bromide, as well as cyanide and other ions which yiel insoluble silver salts in ammoniacal medium, interfere.

Table II. Titration of *n*-Dodecyl Mercaptan in Presence of Chlorid or Bromide

Chloride or Bromide Added	Mercaptan Sulfur Taken	Mercaptan Sulfur Found	
Mg.	*Mg.*	*Mg.*	
100 KCl	1.942	1.941	
300 KCl	1.942	1.947	
1 KBr	1.313	1.307	
2 KBr	1.313	1.306	
5 KBr	1.313	1.413	($AgNO_3$ added rapidly)
10 KBr	1.313	1.378	($AgNO_3$ added slowly)
100 KBr	1.313	1.85	

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Determination of Tungsten in Silicate Rocks

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colorimetric method is described which is suitable for determining ngsten in silicic and mediosilicic rocks for geochemical purposes. s little as 0.5 p.p.m. of tungsten can be detected when a 1-gram mple is taken. The method involves separation of tungsten from on and titanium by sodium hydroxide precipitation, elimination molybdenum as sulfide with antimonic sulfide as collector, and al determination of tungsten with stannous chloride and potassium iocyanate. The yellow thiocyanate of tungsten in a lower valence te is extracted with a small volume of ether.

BECAUSE of the difficulty of determining the minute amounts of tungsten occurring in the common rocks of the earth's ust, relatively little is known concerning its abundance and stribution. Up to now tungsten has been determined by x-ray optical spectrography after concentration by chemical meth-ls. Von Hevesy and Hobbie (*5*), to whom most of our knowl-ge of the abundance of tungsten is due, applied the x-ray ectrographic method to samples ranging in weight from 150 to 0 grams. Wilson and Fieldes (*14*) used an optical spectro-aphic method for the determination of tungsten in schist. In eir procedure tungsten was first concentrated by coprecipita-on with titanium by the joint use of tannin, antipyrine, and cinchonine.

It has been found possible to work out a colorimetric method r tungsten, which gives satisfactory results when applied to icic and intermediate igneous rocks. With subsilicic rocks that ntain much iron and titanium the results are low, approximately e half of the tungsten being lost in the separations. A sample eighing 0.8 to 1 gram is used. As little as 0.5 part per million tungsten can be detected. The method has been used in a udy of the abundance of tungsten in a series of igneous rocks om North America and other parts of the world. The amount the element found in these samples was of the order of a few rts per million, much less than the 69 p.p.m. reported for a mposite of central European rocks (*5*).

Two colorimetric reagents come into consideration for the de-rmination of minute amounts of tungsten—namely; thiocyanate conjunction with a reducing agent such as stannous chloride, d toluene-3,4-dithiol (*7*). Since the latter reagent has so far en used only qualitatively and semiquantitatively, and is dif-ult to obtain, it was decided to use thiocyanate for the present rpose.

OUTLINE OF THE METHOD

The method developed involves four main steps: (1) decom-sition of the sample with hydrofluoric, sulfuric, and nitric acids;) double precipitation of iron, titanium, and other elements th excess sodium hydroxide; (3) precipitation of molybdenum sulfide with antimonic sulfide as collector, in the acidified fil-te from the sodium hydroxide precipitate after the addition of rtrate; and (4) determination of tungsten in the filtrate from e sulfide precipitate by the addition of thiocyanate, hydrochloric id, and stannous chloride, followed by ether extraction of the llow thiocyanate of tungsten in a lower valence state.

DISCUSSION

The important sources of error in the method lie in the copre-itation of tungsten with the precipitate produced by sodium droxide and in the possible incomplete removal of molybdenum sulfide. In the analysis of intermediate and subsilicic rocks e effect of vanadium must be taken into account.

SEPARATION OF TUNGSTEN FROM IRON AND OTHER ELEMENTS. hen extremely small quantities of tungsten are to be determined the thiocyanate–stannous chloride method, appreciable amounts of iron and titanium must be absent. These elements, as well as others incidentally, are separated from tungsten as tungstate by sodium hydroxide precipitation (cf. *11*). In the present case it is necessary to make this precipitation under rather unfavorable conditions as concerns the coprecipitation of tungsten, in that the volumes must be kept small and only a limited excess of sodium hydroxide may be used in order to avoid difficulties later in the procedure. A few preliminary experiments were run to find the approximate amount of tungsten carried down by the hydroxides of ferric iron, titanium, calcium (carbonate), magnesium, and manganese under conditions similar to those that would exist in the determination of tungsten in a silicate rock if a single sodium hydroxide precipitation were made.

In these experiments, 10 ml. of neutral or slightly acid solution containing 5 micrograms of tungsten and the specified amount of metal as chloride (Ca, Mn) or as sulfate (Fe, Ti, Mg) were added dropwise to 15 ml. of hot 10% sodium hydroxide solution. The precipitate was filtered off on a sintered-glass filter crucible and washed with 5 ml. of water. The filtrate was acidified and treated with an excess of hydrochloric acid and stannous chloride to form the yellow tungsten complex, which was extracted with ethyl ether. Comparison was made against a similar ether extract obtained by adding 5 micrograms of tungsten to the filtrate from a like portion of metal solution which had been added to sodium hydroxide. This method of preparation of the standards largely eliminates the effect of any traces of molybdenum possibly present. In the case of calcium, the solution containing tungsten was evaporated to fumes with a slight excess of sulfuric acid to test the recovery of tungsten from slightly soluble calcium sulfate.

The results obtained (Table I) show that there is little tendency for tungsten to be retained by the calcium, magnesium, or manganese precipitate, but that it is coprecipitated with iron and titanium. The loss of tungsten in the sodium hydroxide precipitation is borne out by the results obtained in the application of the method, as finally worked out, to various types of rocks. In spite of the double sodium hydroxide precipitation which is called for in the procedure, recovery of tungsten is not complete when much iron and titanium is present. Useful results (approximately 80% recovery of tungsten) can still be obtained with a sample containing 6% total iron oxides, 0.9% titanium dioxide, 3% magnesia, and 5 or 6% calcium oxide as well as 0.25% phosphorus pentoxide. In other words, the method can be applied to a typical diorite.

As the amount of iron and titanium in the sample increases the loss of tungsten becomes greater, so that only about one half of the tungsten (ca. 4 p.p.m. added) is recovered from a diabase containing 13% total iron oxides and 1.75% titanium dioxide. The proposed method cannot therefore be applied to subsilicic rocks without modification. Presumably better results can be obtained with such rocks by preparing a solution containing approximately the same amount of iron, titanium, etc., as the sample being analyzed and adding an amount of tungsten comparable to that expected in the sample, and then carrying this standard through the procedure. The need for a complete analysis of the specimen

Table I. Recovery of Tungsten in Sodium Hydroxide Precipitation of Iron, Titanium, and Other Elements

(In each case 5.0γ of tungsten were added as tungstate)

Elements Present	W Found
Gram	γ
0.1 CaO	5.0
0.1 MgO	5.0
0.025 MnO	5.3
0.1 Fe_2O_3	4.2
0.1 Fe_2O_3, 0.12 Al_2O_3	4.4
0.015 TiO_2	4.0
0.015 TiO_2, 0.015 P_2O_5	4.0

would be only a slight objection, since the method would usually be applied to analyzed samples. However, the precision probably would not be very good, especially since very low tungsten contents are to be expected with subsilicic rocks, and the method would become considerably more laborious.

With granitic rocks it appears that a single sodium hydroxide precipitation gives good results, but a double precipitation is recommended.

The possibility of precipitating tungsten with α-benzoinoxime, after the addition of molybdenum as a collector, was considered as a possible method for the isolation of tungsten. However, the introduction of molybdenum, which must subsequently be carefully removed before the colorimetric thiocyanate method for tungsten can be applied, is an undesirable feature of such a procedure. Moreover, doubt has been expressed regarding the completeness of precipitation of tungsten by this method (*3*).

Separation of Molybdenum as Sulfide. Molybdenum accompanies tungsten into the filtrate in the sodium hydroxide separation. Before tungsten can be determined by the thiocyanate method, molybdenum must first be removed, since under the conditions it gives a stronger color than does tungsten. The separation of the two elements must be so complete that less than 0.1 microgram of molybdenum will remain with the tungsten. The classical method for the separation of tungsten and molybdenum involves sulfide precipitation of the latter from a dilute mineral acid solution containing tartrate. As is well known, complete precipitation of molybdenum is difficult even with macro amounts (*12*) and would be entirely hopeless for micro amounts unless resort can be made to mixed crystal formation between molybdenum trisulfide and another sulfide possessing slight solubility in acid solution. Extrapolation of known ionic radii (Goldschmidt's) in the series of elements from rubidium to molybdenum on th one hand, and of the elements from silver to antimony on th other, leads to the conclusion that sexivalent molybdenum an quinquevalent antimony should have similar radii. Experimen showed that antimony pentasulfide was actually a good collecto for molybdenum. Trivalent antimony cannot be used in place o the quinquevalent. Moreover, copper sulfide, which has bee recommended as a collector for molybdenum, is not suitable fo the purpose. These findings are in accord with the experi mentally determined or the estimated ionic radii (Å.) Mo^{++++++}, ca. 0.5 (estimated); Sb^{++++}, 0.50 (estimated) Sb^{+++}, 0.90; Cu^{++}, 0.83. (In the nonionic sulfides the radi will be different from these values for the ions, but it may plaus ibly be assumed that the similarity or disparity will be preserve in passing from the ionic type of crystal to the nonionic.)

Preliminary experiments showed that a single hydrogen sulfid precipitation in 25 ml. of hot solution containing 20 micrograms o molybdenum, 1 mg. of quinquevalent antimony, 2 grams o sodium sulfate, and ca. 0.5 ml. of 6 *N* sulfuric acid, left from 0.1 to 0.3 microgram of molybdenum in solution as found by colorimetric examination of the filtrate and washings by the thiocyanate method. When the filtrate from the molybdenum sulfid precipitate was boiled to expel hydrogen sulfide, a small amoun of bromine water added, and the hydrogen sulfide precipitatio repeated after the addition of 1 mg. of quinquevalent antimony less than 0.1 microgram (perhaps less than 0.05 microgram) o molybdenum was found in the filtrate. Thus it appears that on hydrogen sulfide precipitation with antimony as collector does no remove the molybdenum with sufficient completeness for the purpose, but that a second precipitation reduces its concentratio to a negligible value.

In applying this separation of tungsten and molybdenum to silicate rocks, the effect of phosphorus must be considered. The precipitation of molybdenum becomes more difficult when phosphate is present because of the greater ease of reduction of molybdenum by hydrogen sulfide (*12*). It is not advisable to make the hydrogen sulfide precipitation from an initially hot solution when appreciable amounts of phosphorus are present, as is the case with basic rocks. Hydrogen sulfide should be passed into a cold solution to minimize the reduction of molybdenum to molybdenum blue. The colloidal suspension of the sulfides may then be heated to hasten coagulation. This procedure satisfactorily separated the molybdenum from a diabase containing 0.25% of phosphorus pentoxide. It is believed that two precipitations with hydrogen sulfide as described in the procedure below are sufficient to remove with satisfactory completeness the quantities of molybdenum likely to be encountered in igneous rocks. The maximum amount of molybdenum found in a series of 22 American rocks was 0.0007%, or 7 micrograms in a 1-gram sample (*10*). The presence of even less than 5 micrograms of molybdenum in the antimony sulfide precipitate (1 mg. of antimony) is made evident by a pronounced change in color of the precipitate from orange to brownish.

The second antimony sulfide precipitates obtained from some rock samples of intermediate silica content which contained about 1 p.p.m. of molybdenum were examined for molybdenum by dissolving in sodium hydroxide, adding excess bromine and warming, and finally treating with thiocyanate and stannous chloride in excess hydrochloric acid, followed by extraction with a small volume of ethyl acetate. In some cases no molybdenum could be detected, in others 0.1 to 0.2 microgram was found.

Determination of Tungsten. After concentration of the filtrate from the last sulfide precipitate to expel hydrogen sulfide and to reduce the volume to a convenient size, the solution is extracted with ethyl ether to remove traces of a foreign coloring substance (not identified but possibly from the bromine) which imparts a faint brownish coloration to the solvent. The extracted solution is treated with potassium thiocyanate and stannous chloride in strong hydrochloric acid to form the thiocyanate com-

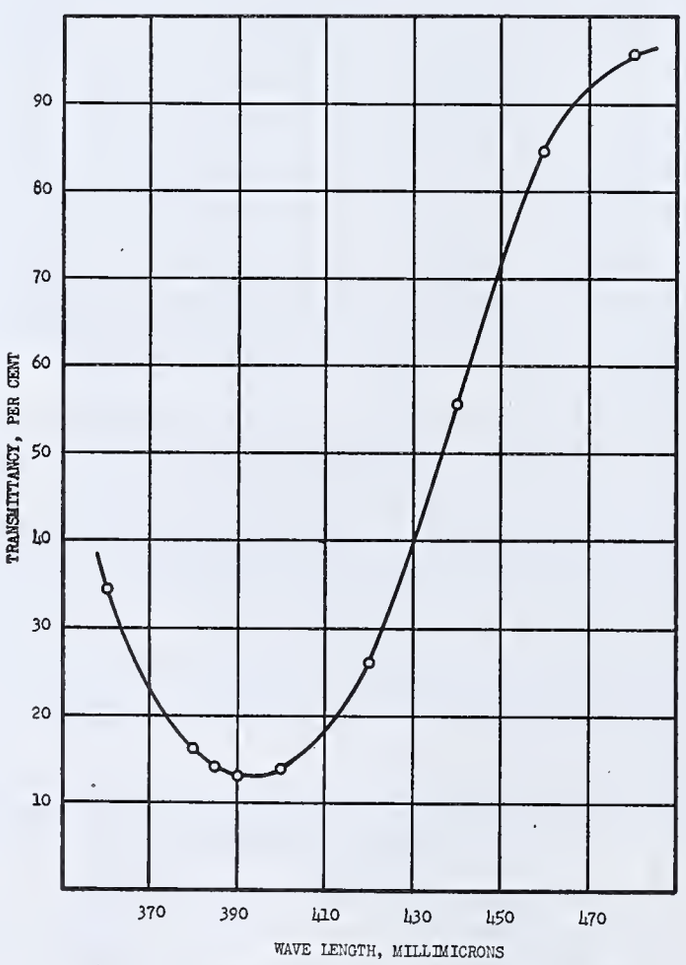

Figure 1. Transmission Curve of Tungsten-Thiocyanate Complex in Ethyl Ether

40 p.p.m. of tungsten, 1-cm. cell
Curve constructed from data obtained by B. Warshowsky using Coleman Model 11 spectrophotometer with 35-millimicron slit width. Color developed by Feigl's method (*2*) and complex extracted after 2 hours

Table II. Determination of Tungsten in Silicate Rocks

Sample	Sample Weight	W Present	W Added	W Found	W Recovered
	Gram	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*
ynthetic silicic rock[a]	1	0.7	3.0	3.3	2.6
ynthetic silicic rock[a]	1	0.7	5.0	6.4	5.7
ynthetic silicic rock[a,b]	1	0.7	10.0	9.0	8.3
ynthetic silicic rock[a]	1	0.7	10.0	10.6	9.9
ynthetic silicic rock (10 p.p.m. Mo)[a,b]	1	0.7	10.0	12.7	12.0
ynthetic silicic rock (10 p.p.m. Mo)[a]	1	0.7	10.0	10.8	10.1
ynthetic silicic rock (15 p.p.m. Mo, 300 p.p.m. V_2O_3)[a]	1	0.7	5.0	6.2	5.5
ynthetic silicic rock (20 p.p.m. Mo)[a]	1	0.7	0	1.2	(0.5)
ranodiorite	1	1.1	2.0	3.2	2.1
ranodiorite[a]	1	1.1	3.0	3.5	2.4
ranodiorite	1	1.1	3.0	3.7	2.6
ranodiorite (11 p.p.m. Mo)[a]	1	1.1	0	1.2	(0.1)
uartz monzonite	0.8	1.5	2.5	3.5	2.0
iorite A	0.8	1.6[c]	3.7	4.6	3.0
iorite B	0.8	0.9[c]	3.7	3.8	2.9
iabase	0.8	1.0[c]	3.7	2.9	1.9
iabase	0.8	1.0[c]	6.2	5.0	4.0

[a] Single NaOH precipitation instead of double as in others.
[b] One H_2S precipitation instead of two as in others.
[c] Not corrected for vanadium.

lex of tungsten in a lower valence state (*2*, *4*). It is not necesary to add these reagents to an initially basic solution as in previus procedures for determination of tungsten by the thiocyanate ıethod. This colorimetric method for tungsten has not been tudied in a systematic manner but it is known that a high acid oncentration is necessary and that the color develops slowly at oom temperature, so that the solution should be allowed to stand or an hour. Beer's law is obeyed. The color reaches its maxiıum intensity more rapidly if the solution is warmed (*8*), but too igh a temperature or too long a period of heating results in the ormation of a small amount of stannous sulfide. In the present ork the colored compound was allowed to develop at room temerature.

The tungsten complex is then extracted with a small volume of ther. Without extraction the determination would be impossible because of the very minute amounts of tungsten involved. Moreover, the aqueous solution may show a slight coloration due o vanadium and possibly other substances. The tungsten conent of the ether extract is found by comparison against a series f standards, or against a single standard by varying the height f the column of solution in a narrow tube. The visual comparion is preferred to a photometric measurement of color intensity, ecause of the very faint color obtained ith the quantities of tungsten normally resent in igneous rocks. The visual comarison can readily be made in small glass ubes having a cross-sectional area of sq. cm. Under the conditions described elow 0.3 microgram of tungsten imparts faint but definite coloration to the ether ı such a tube—i.e., the sensitivity of the eaction is 0.3 microgram of tungsten per quare centimeter. The photometric sentivity may be expressed in terms of the uantity of tungsten in a column of ether solution having a crossectional area of 1 sq. cm. which will produce a barely measurable xtinction at the wave length of maximum absorption. The transission curve of the tungsten thiocyanate complex in ether, obained under conditions similar to, but not identical with, those of he procedure below is shown in Figure 1. It is evident from this urve that at approximately 390 mμ, an extinction of 0.001 corresponds to about 0.05 microgram of tungsten in a column of soluon having a cross section of 1 sq. cm. This is the photometric ensitivity based on 0.001 as the smallest extinction ($\log I_0/I$) that an be measured. In other words, visual comparison is roughly s sensitive as photometric measurement in an absorption cell of -cm. thickness when the volume of the solution is 5 ml.

Only one ether extraction of the aqueous solution is made. The partition coefficient of the tungsten complex is such that the latter is concentrated in the ether even when the volume of the aqueous phase is much greater. Any error due to unextracted tungsten is cancelled by treating the standard solution in the same manner. The salt solution used to prepare the aqueous standard solution is treated with hydrogen sulfide to remove any traces of molybdenum that may be present. The error in comparing a solution containing 1 microgram of tungsten with a standard of similar concentration may amount to ±0.3 microgram, but is less on the average.

Comparatively few elements will survive the sodium hydroxide and hydrogen sulfide precipitations and find their way into the final aqueous solution. Chief among these, which may have an effect on the determination of tungsten, are phosphorus and vanadium. Phosphate (10 mg. of phosphorus pentoxide) was found to have no effect. Borate also did not interfere. Vanadium, however, produces a coloration similar to that given by tungsten under the conditions of the determination. Hoffman and Lundell (*6*) have already observed that vanadium interferes to a slight extent in the analogous determination of molybdenum with thiocyanate (ethyl ether as extractant) by giving a color about $^1/_{200}$ as strong as molybdenum. In the procedure described below 500 micrograms of vanadium sesquioxide produce as much color as 1 ± 0.2 microgram of tungsten. The hue of the vanadium compound is somewhat more brown than that of the tungsten complex in ether solution, but the two are not readily distinguishable at low concentrations. The amount of vanadium in silicic rocks is too small to affect the determination of tungsten by a detectable quantity. In intermediate rocks it is possible that enough vanadium may at times be present to give an appreciable positive error. Thus a sample containing 0.03% of vanadium sesquioxide would show an approximate apparent tungsten content of 0.6 p.p.m.

Although a vanadium oxide content of 0.03% is not likely to be encountered in rocks for which the proposed method is intended, the possibility of error from the presence of vanadium must be borne in mind. The vanadium content of the sample can be determined on a separate portion (*9*). Then, if the quantity of vanadium in the sample warrants, the equivalent amount of vanadate can be added to the standards to compensate the color produced in the sample solution. The possibility of determining vanadium directly in the sample solution, prior to the addition of thiocyanate and stannous chloride, by adding a little hydrogen peroxide was considered, but this does not seem to be a desirable procedure because of the common presence of a trace of iron, and possibly of titanium, in this solution.

Table III. Composition of Samples in Table II

	SiO_2	Al_2O_3	Fe_2O_3	FeO	MgO	CaO	Na_2O	K_2O	TiO_2	P_2O_5	MnO
Synthetic silicic rock	77	17	2.1	...	1.3	2.2	...	...	0.2	0.1	0.1
Granodiorite	68.2	15.2	0.6	3.3	0.8	2.0	3.4	5.1	0.67	0.23	0.08
Quartz monzonite	65.6	16.6	1.3	2.5	1.1	3.4	4.1	3.7	0.57	0.17	0.08
Diorite A	62.6	16.3	1.3	4.0	2.7	5.3	3.5	2.6	0.89	0.21	0.08
Diorite B	61.5	15.5	2.5	3.6	3.1	5.7	3.4	1.8	0.88	0.26	0.14
Diabase	52.7	14.5	7.4	5.6	3.7	8.0	3.2	1.1	1.76	0.25	0.24

With the exception of vanadium, no element that can be present is known to give a coloration with thiocyanate and stannous chloride in the final solution obtained according to the procedure described below. Any rhenium escaping separation in the preliminary treatment would produce a color resembling that of molybdenum in the final solution, but since the amount of rhenium in igneous rocks is of the order of 0.001 p.p.m., its effect can be left out of account. Columbium and tantalum are without effect (*13*). Uranium in small amount was found to impart no color to the ether phase. Titanium in small amount together with phosphate gave no color. Fluoride in comparatively large amounts tends to decrease the tungsten color intensity, but it will not be present.

A small amount of iron is always present in the filtrate from the sodium hydroxide precipitation, apparently in colloidal form. Frequently more iron is present in the filtrate from a silicic rock than in that from a subsilicic one. The iron thus introduced imparts a yellowish color to the acid tartrate solution but does not interfere in the determination of tungsten. One milligram of Fe_2O_3 intentionally added produced no coloration in the ether. However, in some of the determinations the ether solution of the tungsten complex darkened slightly on long standing. This darkening, with a change in hue to brownish, may possibly be due to the presence of iron. No error results if the color comparison is made immediately after extraction. It was observed that the color of an old extract could be restored to the original hue and intensity by adding a few drops of 10% stannous chloride in concentrated hydrochloric acid to the tube together with an equal volume of water and shaking well.

Platinum, introduced in traces as a result of attack in the decomposition, might be expected to interfere by being reduced to chloroplatinous acid, soluble in ether with a yellow color, if not completely removed as sulfide. The possibility of error from this source was investigated by carrying a sample of granodiorite through the procedure as described below, except that thiocyanate was omitted in the final colorimetric determination. The ether extract was not perceptibly colored; it could not be distinguished from a blank. The tungsten complex was formed when thiocyanate was added to the extracted aqueous solution.

When the tungsten content of the sample is greater than 2 p.p.m., the hue of the ether extract offers considerable evidence that the coloration is actually due to tungsten and not to some other substance. The tungsten complex has a characteristic pure yellow color easily distinguishable from the amber of the molybdenum thiocyanate.

The possibility of using ethyl acetate instead of ether as an extractant for the tungsten thiocyanate complex was investigated, but it was found that the former solvent was less suitable because it was more strongly colored by vanadium (and molybdenum) than was ethyl ether under the conditions of the determination. It is possible that pure isopropyl ether can be used in place of the more volatile ethyl ether. Isopropyl ether has been used by Cunningham (*1*) as an extractant for the tungsten thiocyanate complex in procedures for the determination of tungsten in low-grade ores and tailings.

No tungsten was found in the reagents.

SPECIAL SOLUTIONS

Sodium Hydroxide, 10 grams in 100 ml. of water.

Tartaric Acid, 50 grams in 100 ml. of solution.

Antimony Pentachloride, 0.5% antimony in 4 *N* hydrochloric acid. This solution may be prepared by adding saturated potassium bromate solution to a hot antimony trichloride solution containing 0.5% antimony in 4 *N* hydrochloric acid until the color of bromine appears.

Potassium Thiocyanate, 10 grams in 100 ml. of water.

Stannous Chloride, 5 grams of the dihydrate in 100 ml. of concentrated hydrochloric acid. The solution should not be older than a month.

Ethyl Ether. Only the reagent grade product should be used. The best quality ether need not be subjected to any purification procedure before use.

Salt Solution for Standards. Dissolve 50 grams of sodium hydroxide and 10.0 grams of tartaric acid in 250 ml. of water and add to the cold solution 225 ml. of 6 *N* sulfuric acid and 10 ml. of antimony pentachloride solution. Pass a rapid stream of hydrogen sulfide into this solution, cooled to room temperature, for 0.5 hour. Allow the mixture to stand overnight and filter off the precipitate on a retentive paper.

Standard Tungsten Solution, 100 micrograms of tungsten per milliliter. Prepare by dissolving pure sodium tungstate dihydrate in water and diluting to volume $\left(\frac{Na_2WO_4.2H_2O}{W} = 1.79\right)$.

COLOR COMPARISON TUBES

These should have a capacity of 5 ml. with the dimensions 1.2 × 8 cm., and should be provided with glass stoppers. A narrow strip of millimeter graph paper is glued to the back of each tube to serve as a scale for reading the height of the liquid column. The paper strip should be waterproofed with paraffin or other suitable material.

PROCEDURE

Transfer 1.0 gram of 100-mesh silicic rock or 0.8 gram of medium silicic rock to a platinum dish and add 5 ml. of 6 *N* sulfuric acid, 2 ml. of concentrated nitric acid, and 5 ml. of 48% hydrofluoric acid. Evaporate to dryness and heat the residue until fumes of sulfuric acid cease to come off. Add 1 ml. of 6 *N* sulfuric acid and a few milliliters of water to the dish and warm with stirring to bring as much as possible of the salts into solution. Then evaporate to dryness and fume off the excess of sulfuric acid at approximately 350° C. Add 1 ml. of 6 *N* sulfuric acid and 10 ml. of water and digest slightly below the boiling point for 15 or 30 minutes while stirring at intervals. Disregard undissolved material.

Sodium Hydroxide Precipitation. Add the mixture dropwise with stirring to 15 ml. of hot sodium hydroxide solution in a 50-ml. beaker. Pour the liquid back into the platinum dish and digest slightly below the boiling point for about 15 minutes. Filter the mixture through a sintered-glass crucible (Jena 1G4 or equivalent), catching the filtrate in a vial or test tube hung inside the suction flask. Wash the precipitate, which has been sucked dry, with 2 or 3 ml. of water. Transfer the filtrate to a 50-ml. beaker and place on a steam bath to reduce the volume by evaporation.

By means of a stirring rod transfer most of the precipitate in the crucible to the beaker in which the precipitation was made. Clean the platinum dish with about 5 ml. of 1 to 1 hydrochloric acid and pour the solution into the crucible. Without applying suction, allow the precipitate remaining in the crucible to dissolve and then pour the solution into the beaker containing the solution of the major part of the precipitate. Now apply suction and wash the crucible with a few milliliters of water. Add the washings to the solution of the precipitate and evaporate the whole to a volume of a few drops. Dilute with 5 ml. of water and add the solution dropwise to 10 ml. of hot sodium hydroxide solution. Keep the mixture slightly below the boiling point for 10 or 15 minutes and then filter through a sintered-glass crucible. Wash with two portions of water totaling 5 ml. Combine the filtrate and washings with those from the first precipitation and evaporate the whole to a volume of 15 ml.

Hydrogen Sulfide Precipitation. Cool the evaporated solution to room temperature and add 6 *N* sulfuric acid dropwise with stirring until a slight permanent precipitate of aluminum hydroxide is formed. Then add 1.0 ml. of tartaric acid solution, 0.5 ml. of 6 *N* sulfuric acid, and 0.2 ml. of antimonic chloride solution. If necessary allow the solution to stand until it becomes clear. Cover the beaker and pass in a rapid stream of hydrogen sulfide for 10 minutes. Then heat the solution to the boiling point while continuing the passage of hydrogen sulfide, keep at the boiling point for a few minutes, remove the beaker from the heat, and pass in the gas for another 5 or 10 minutes. Stirring during this interval aids in the coagulation of the precipitate. Allow the precipitate to stand for 2 hours or preferably overnight.

Filter off the sulfide precipitate on a small (5-cm.) filter paper of fine texture and wash with a few milliliters of hydrogen sulfide water containing a drop of 6 *N* sulfuric acid. Evaporate the combined filtrate and washings to 15 ml. Cool the solution to room temperature and add 5 drops of bromine water (a yellow color should persist for a minute or two) and 0.2 ml. of antimonic chloride solution. Precipitate with hydrogen sulfide as before. Allow the precipitate to stand overnight and filter it off on a small retentive filter paper which has been washed with a little dilute (0.5 *N*) sulfuric acid. Wash the precipitate with 2 or 3 ml. of cold water containing a drop of 6 *N* sulfuric acid.

Determination of Tungsten. Evaporate the combined filtrate and washings from the second sulfide precipitation to a volume of 15 ml. in a 50-ml. beaker. At the same time evaporate 25-ml. portions of the salt solution for the standards to which have been added 1, 2, and 3 micrograms of tungsten, respectively. If the vanadium content of the sample is equal to, or greater than, 0.02% (0.03% vanadium sesquioxide), add an amount of ammonium metavanadate equivalent to the quantity of vanadium in the sample to each of the standards (see Discussion, p. 163).

Cool each solution to room temperature (20° C.), transfer to a small separatory funnel, and extract with 5 ml. of ether. Draw off the aqueous solution in each case and wash the ether layer in the funnel with 1 or 2 ml. of water, adding the latter to the extracted aqueous solution. If the ether extract from the sample solution shows an appreciable coloration (compare in a small vial against the ether from one of the standard solutions), extract the aqueous solution once more with 2 to 3 ml. of ether. Discard the ether extracts.

Treat the extracted sample and standard solutions with 1.0 ml. of potassium thiocyanate and 10 ml. of stannous chloride solution. If necessary adjust the volumes to equality by adding water until the heights of the solutions in the beakers are the same. The volume should lie between 25 and 30 ml.; if it is less than 25 ml., salt is almost certain to crystallize out. After mixing, set the solutions aside at 25° to 30° C. for an hour.

Cool the solutions to 20° C. or slightly less and transfer to separatory funnels. Extract each solution with 5.0 ml. of ether by shaking for 15 seconds. Allow the phases to separate, drain off the aqueous solutions, and run the ether layers into the color comparison tubes. If desired the funnels can be rinsed with 1 ml. of ether. Compare the tubes axially against a white background in good light. Pick out the standard having a color intensity closest to the sample. By means of a glass tube remove small volumes of ether from the standard or the sample tube, as the case requires, until the two solutions show the same color intensity. Usually it is best to note the heights of the solution being withdrawn at which it is just perceptibly darker and lighter, respectively, than the solution against which comparison is made. The intermediate height is then taken as the matching height. This operation should be carried out in a cool environment (20° C.) to avoid undue loss of ether by evaporation. Find the tungsten content of the sample from the ratio of the matching height to the original height of the solution whose depth is varied. Run a blank through all steps of the procedure.

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Equations of Thixotropic Breakdown for the Rotational Viscometer

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Fundamental principles of the thixotropic hysteresis loop and equations of thixotropic breakdown as produced by the rotational viscometer are presented. The work has practical applications in printing and in all other types of coatings where changes in consistency may affect the quality of the manufactured product. The equations developed give the relationship between consistency and rate or duration of shear applied to the material and enable calculation of plastic viscosity and yield value for any rate of shear that is desired.

IN A previous paper (*2*) on thixotropy, it was experimentally shown (with pigment-vehicle suspensions) that breakdown can be induced in two ways—the "breakdown by time" and the "breakdown by rate of shear". The first is exemplified by applying a constant rate of shear for a sufficient time. Breakdown will continue until all bonds below a certain strength are broken and equilibrium is attained. The second way is employed when further breakdown is required. In this case, it is necessary to increase the rate of shear, making more power available; then stronger bonds are broken.

In the authors' work on thixotropy, the rotational viscometer (*1*) has been used exclusively. There are several reasons for doing this: The material under test is not lost by extrusion, but remains in the viscometer cup; hence the results of increasing breakdown can be continuously recorded; the rate of shear employed can be regulated by adjusting the r.p.m.; and the Reiner and Riwlin equation (*3*) can be applied for determining plastic viscosity and yield value.

The consistency curve obtained with the rotational viscometer is composed of an upcurve and a downcurve. These curves do not coincide when the material is thixotropic, thus forming a "hysteresis" loop, used for measuring thixotropic breakdown. It is not formed when the measurements are obtained with an extrusion viscometer.

Assuming as previously (*2*), that breakdown is proportional to the top rate of shear it has been shown that

$$e^{\frac{2U}{M}} = k/\omega^2 \qquad (1)$$

where U is the plastic viscosity, ω the top angular velocity, k a constant for the upcurve of the loop, and M a coefficient of thixotropic breakdown. An analysis (*2*) of the mathematical procedure leading to M will show that it is the loss in shearing force per cm.^{2} per unit increase in rate of shear.

Because no action can take place in zero time, it follows that if ω is raised to $\omega + d\omega$, a time dt is involved. The coefficient M, therefore, is in some way associated with time. It is important that the exact nature of this association be understood, because the investigator would like to know if M is an attribute only of the test material, or whether its value also depends on the method of carrying out the measurement.

When a constant rate of shear is applied to a thixotropic material, the plastic viscosity decreases with the logarithm of the time. This has been shown by Weltmann (*5*), and has been expressed as:

$$B = -\frac{dU}{dt} \times t \qquad (2)$$

B is a constant and is called the "time coefficient" of thixotropic breakdown. In order to show the relation between M and time, it is necessary only to combine Equations 1 and 2. Before doing this, it should be explained more fully what is meant by "time".

If an unbroken material is placed in the viscometer and a constant shearing force applied resulting from an angular velocity ω, the material will break down with a rate B/t as given in Equation 2. If the torque at point A, Figure 1, is the initial and therefore maximum torque acquired for an angular velocity ω, then after a certain time, t, point C will be reached because the material is steadily breaking down. If fresh material of the same kind as before is now used and the upcurve, starting at point B, is made, it will be found that when the time is correctly adjusted, the curve will meet point C in exactly the same time, t, which it took when starting from A. If the time of the upcurve is adjusted too short, the curve will meet some point C': if too long, some point C''. The conclusion is that the time needed to attain a certain broken down state such as exists at C is the same regardless of the path taken—i.e., from A to C or from B to C. This means that time t in Equation 2 when going

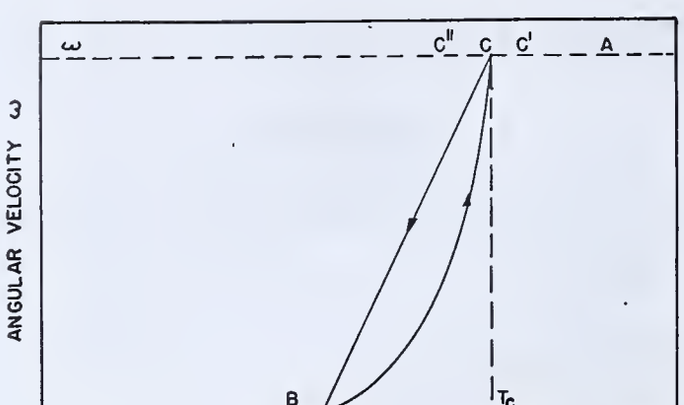

Figure 1. Schematic Flow Curve

from A to C is the same as the time of the upcurve of the hysteresis loop implied in Equation 1, when going from B to C.

In obtaining the upcurve a definite number, N, of experimental points are made. To each point is given the same amount of time (Δt). Adjacent points are separated by the same number of r.p.m. Hence $t = \Sigma(\Delta t)$ and is proportional to ω. Consequently,

$$t = R\omega \tag{3}$$

and

$$\Delta t = t/N \tag{4}$$

where R is the proportionality constant.

SYMBOLS USED IN PAPER

A. Area of hysteresis loop
A_E. Area of equilibrium hysteresis loop
B. Coefficient of thixotropic breakdown with time
C. Rotational viscometer constant for calculating f
Δt. Time element for each point on curve
f. Yield value
f', f''. Yield values at different top rates of shear
h. Depth of immersion of bob
J. Slope of the yield value curve, f *vs.* $(\omega - \omega_0)$
K. Proportionality constant. Independent of Δt
k. Proportionality constant. Not independent of Δt
k_0. Value of k when M_0 takes the place of M. Independent of Δt
k'. Value of k when ω_0 is finite. Not independent of Δt
k''. Proportionality constant in yield value curve, f *vs.* $(\omega - \omega_0)$. Independent of Δt
M. Coefficient of thixotropic breakdown for increasing ω. It contains B, which being constant permits M to be independent of time
M_0. That part of M associated only with rate of shear. Independent of Δt
N. Number of points on upcurve
ω. Angular velocity
ω_0. Angular velocity of crossing point
R. Proportionality constant between ω and t
R_b. Radius of bob
R_c. Radius of cup
RPM. Revolutions per minute at top point of curve
rpm. Revolutions per minute
$(rpm)_0$. Revolutions per minute at crossing point
S. Rotational viscometer constant for determining U
t. Total time in reaching top point of curve
t_E. Total time in reaching top point in equilibrium curve
T. Torque
T_E. Torque at top point of equilibrium curve
T_2. Torque of yield value intercept
T_{ω_0}. Torque at crossing point
V. Coefficient of thixotropic breakdown when yield value intercept is not constant. Independent of Δt

INTRODUCTION OF TIME

Returning to the addition of Equations 1 and 2.

From what has been stated, it is reasonable to assume that M is composed of two factors, the one factor arising from increasing the rate of shear (expressed by an increase, $d\omega$, in the angular velocity); and the other resulting from the duration of the shearing force (equal to dt for the increase $d\omega$). Let M_0 be the part of M associated only with an increase in ω. Then making the same basic assumption as made in the previous paper, that the thixotropic loss in torque (the torque that corresponds to the loss in shearing force) is proportional to the rate of shear, it follows that

$$e^{2U_\omega/M_0} = k_0/\omega^2 \tag{5}$$

where M_0 and k_0 have values that differ from the previous M and k; and U is designated at U_ω to distinguish it from the U determined under normal conditions. From Equations 5 and 2,

$$dU_\omega = -M_0 d\ln\omega \tag{6}$$

$$dU_t = -B d\ln t \tag{7}$$

Adding gives

$$dU_\omega + dU_t = dU = -M_0 d\ln\omega - B d\ln t \tag{8}$$

where U_t is the change in U induced only by the duration of the shearing force.

Integrating gives

$$U = -M_0 \ln\omega - B\ln t + \ln K^{M_0/2} \tag{9}$$

where $\ln K^{M_0/2}$ is the constant of integration. K is given the exponent $M_0/2$ so that it will be dimensionally correct. The introduction of M_0 is permissible because M_0 is independent of t and ω.

Condensing 9 gives

$$e^{2U/M_0} = K/\omega^2 t^{2B/M_0} \tag{10}$$

Our hypothesis in regard to the dual nature of M specifies that $M = M_0 + X$, where X is some unknown factor related to time. The next step is to find what X actually is. Using two points, $(U_1\omega_1)$ and $(U_2\omega_2)$, it can be shown with the aid of Equation 10 that

$$M_0 = 2(U_1 - U_2)/\ln(\omega_2^2/\omega_1^2) - B[\ln(t_2^2/t_1^2)/\ln(\omega_2^2/\omega_1^2)] \tag{11}$$

It has been previously established (*2*) that

$$2(U_1 - U_2)/\ln(\omega_2^2/\omega_1^2) = M \tag{12}$$

Substituting 12 in 11 and making $t = R\omega$ (see Equation 3) gives

$$M = M_0 + B \tag{13}$$

The unknown quantity, X, is therefore B, the time coefficient of thixotropic breakdown.

Substituting Equation 13 in Equation 10 gives

$$e^{2U/(M-B)} = K/\omega^2 t^{2B/(M-B)} \tag{14}$$

and

$$e^{2U/M} = K^{(M-B)/M}/\omega^{2(M-B)/M} t^{2B/M} \tag{15}$$

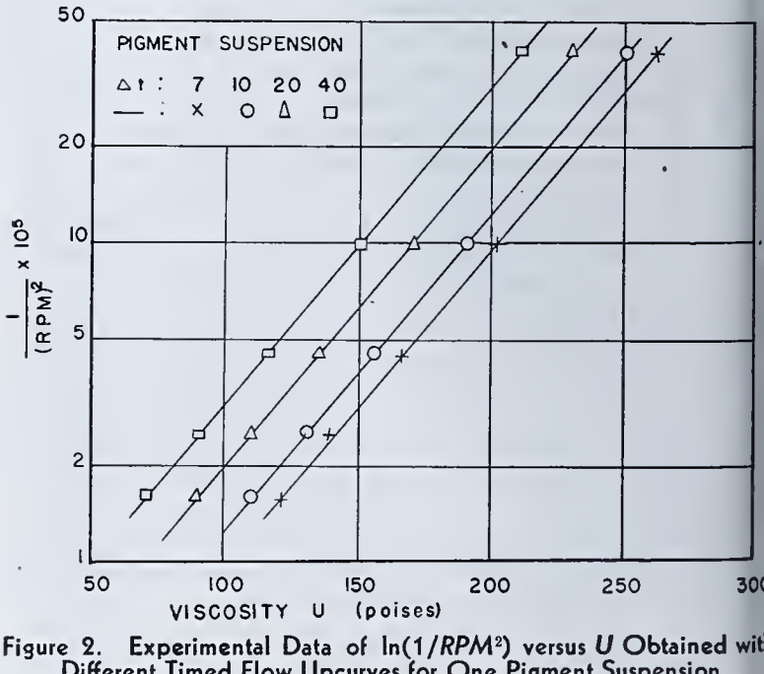

Figure 2. Experimental Data of $\ln(1/RPM^2)$ versus U Obtained with Different Timed Flow Upcurves for One Pigment Suspension

EXPERIMENTAL PROOF OF INDEPENDENCE OF M AND (Δt)

From Equation 1 it is evident that when U is plotted against $\ln(1/\omega^2)$, a straight line results with a slope equal to $2/M$. A change in (Δt) moves the straight line parallel with itself, thus maintaining the same slope. This means that M does not vary and is independent of (Δt). The experimental evidence of this has been obtained for various pigment suspensions. The curves for one of these suspensions is shown in Figure 2.

EFFECT OF TIME ON UPCURVE AND ON AREA EQUATIONS

Plastic viscosity is

$$U = (T - T_2)S/\omega \tag{16}$$

Substituting 16 in 15 gives the equation of the upcurve:

$$T = \frac{(M - B)\omega}{2S}\ln K - \frac{(M - B)\omega}{S}\ln\omega - \frac{B\omega}{S}\ln t + T_2 \tag{17}$$

It has been shown (*2*) that the area of the hysteresis loop is

$$A = M\omega^2/4S \tag{18}$$

Since M is independent of (Δt), the area is not affected by any change in (Δt). This is not true of the equilibrium hysteresis loop. The equilibrium loop is made by allowing the top shearing force to act until equilibrium is attained. The downcurve is then made. Referring to Figure 3, the normal loop is $ADBA$; the equilibrium loop is $ADBCA$. Its area will be designated as A_E and the equilibrium time as t_E. The time of the upcurve, ADB, is t.

The area of the normal loop, as before, is A. Then

$$A_E = A + (T - T_E)\omega/2 \tag{19}$$

Also

$$T_E = \frac{(M - B)\omega}{2S}\ln K - \frac{(M - B)\omega}{S}\ln\omega - \frac{B\omega}{S}\ln t_E + T_2 \tag{20}$$

Subtracting Equation 20 from Equation 17 gives

$$T - T_E = \frac{B\omega}{S}\ln(t_E/t) \tag{21}$$

Substituting Equations 18 and 21 in Equation 19 gives

$$A_E = \frac{M\omega^2}{4S}\left(1 + \frac{2B}{M}\ln(t_E/t)\right) \tag{22}$$

Since ω, M, S, B, and t_E are constants, Equation 22 is of the form

$$A_E = a - b\ln t \tag{23}$$

Equation 23 gives a linear curve with a negative slope when A_E is plotted against $\ln t$.

In the authors' initial work on the loop area, it was impossible to determine from experimental data whether the abscissa should be t or $\ln t$. Both forms seemed to give a linear relationship with the loop areas. The shape of the curve was not noticeably affected by shifting from t to $\ln t$, so no decision could be made as to which expression was correct. Equation 22 was not then available as a guide; so the simpler of the two forms was selected and the abscissa was plotted as t. The experimental difficulties in obtaining a large series of equilibrium loops on a single thixotropic material are not small; consequently, the experimental points deviate somewhat from their linear position, making a double decision possible. In the original paper (*2*) the laboratory measurements indicated that curve A, Figure 4, made a small angle with the horizontal. Since t does not affect the area of the normal loop, it is evident that this small angle should have been zero. Subsequent data have shown that this small angle is within the limit of experimental error.

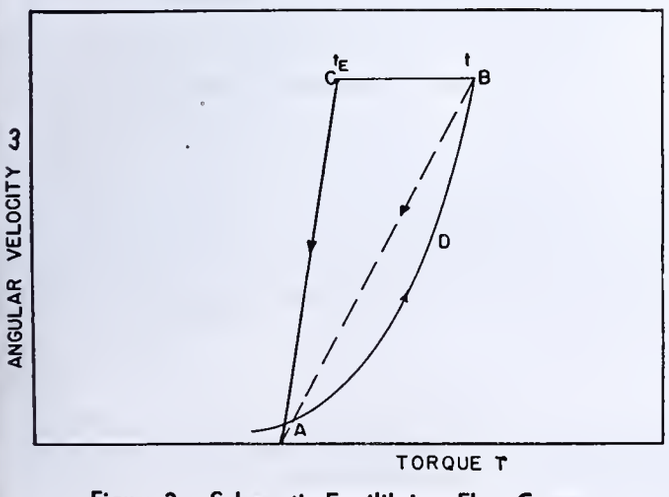

Figure 3. Schematic Equilibrium Flow Curve

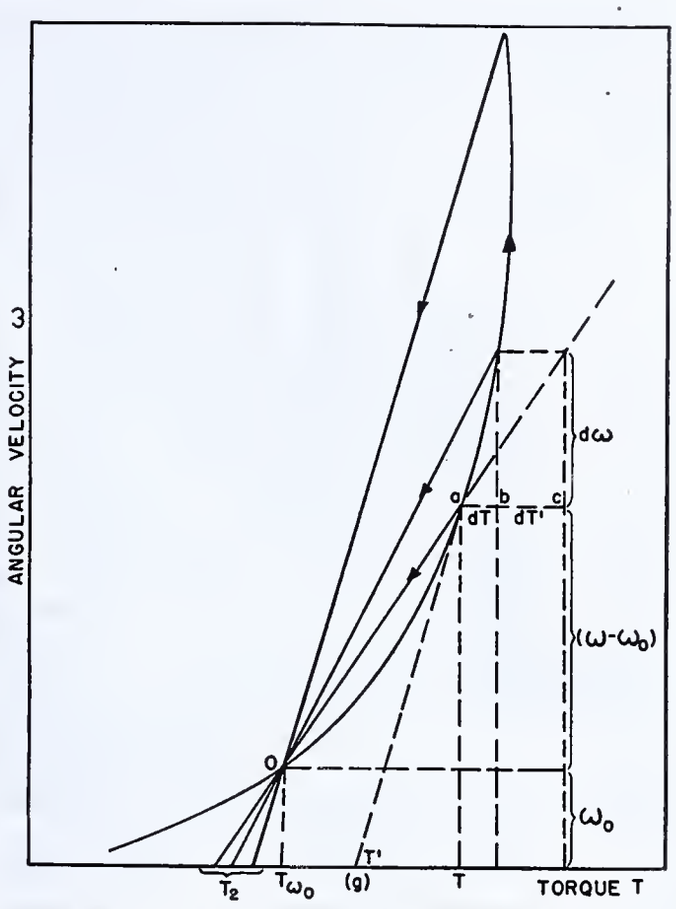

Figure 4. Schematic Flow Curve Demonstrating a Common Crossing Point at ω_0 for Different Downcurves

DIFFERENCE BETWEEN k AND K

When a thixotropic material breaks down, it does so in accordance with Equations 1 and 15.

Let it be assumed that breakdown can be continued until U actually becomes equal to zero. It can then be seen that k and K are equal to the squares of the theoretically highest angular velocities. These two constants do not behave alike. In Equation 1, a change in (Δt) cannot affect M or B. Such a change, therefore, must alter the value of k. This it does, as can be seen from the different intercepts of the series of curves given in Figure 2. In Equation 15, t is included; so any variation in (Δt) affects t and thus allows K to remain constant.

A direct comparison of k and K can be made. Substituting $t = R\omega$ in Equation 14; then

$$e^{2U/(M - B)} = K/(R\omega)^{2B/(M - B)\omega^2} \tag{24}$$

and

$$e^{2U/M} = K^{(M - B)/M}/R^{2B/M}\omega^2 \tag{25}$$

Comparing Equation 25 with Equation 1 shows that

$$k = K^{(M-B)/M}/R^{2B/M} \tag{26}$$

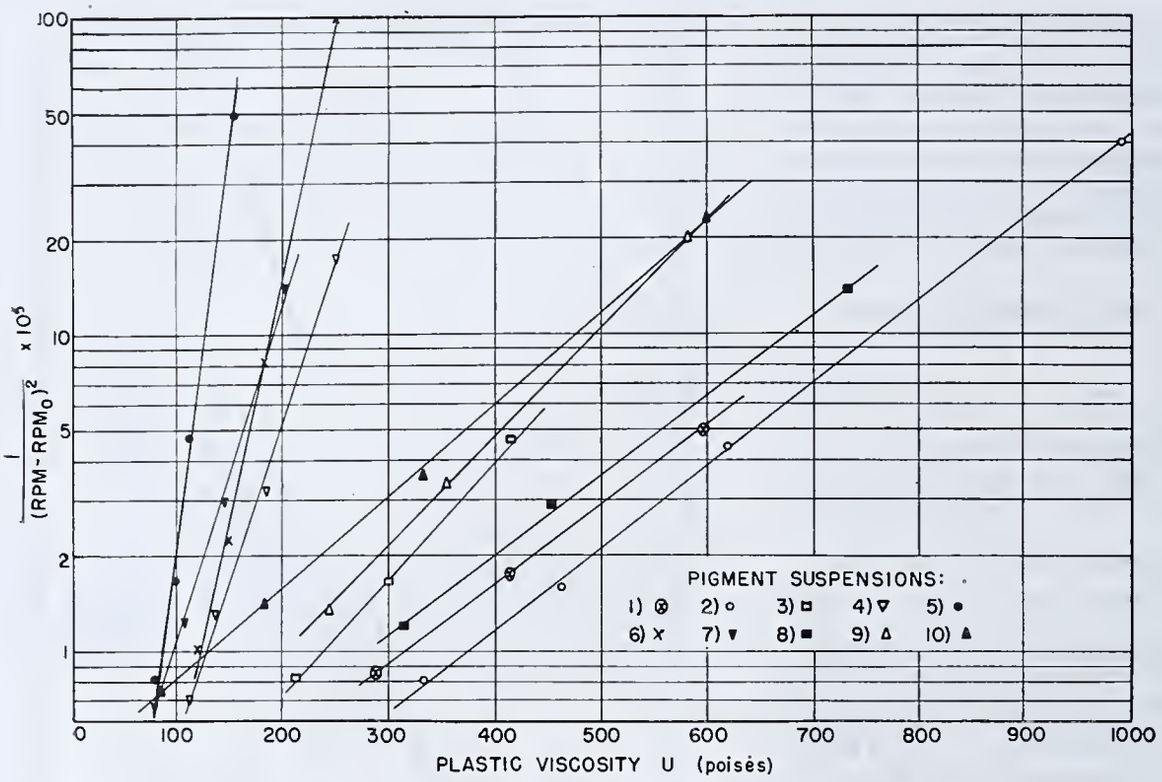

Figure 5. Experimental Data of $\ln[1/(RPM - rpm_0)^2]$ versus U for Various Pigment Suspensions

The preceding work has been developed for the purpose of showing that M and B define that property of a thixotropic material that regulates its method and extent of breakdown. Neither M nor B is a "coefficient of thixotropy", for that term refers to a property that cannot be expressed dimensionally. It falls in the same category with concepts like "toughness", "brittleness", and "hardness". On the other hand, thixotropic breakdown is a physical reaction subject to measurement. M gives the drop in plastic viscosity resulting from a unit increase in rate of shear. M has been shown in this paper to be independent of the speed at which the upcurve is made, and therefore belongs entirely to the material. It does not depend on the dimensions of the viscometer or on the method of measurement being applied. B, likewise, has been shown to be independent of the instrument and of time.

CHANGE IN YIELD VALUE INTERCEPT WITH THIXOTROPIC BREAKDOWN

In the authors' previous paper on thixotropy, the decrease in plastic viscosity resulting from thixotropic breakdown was described. No mention was made at that time of any corresponding change in the yield value intercept because such an effect was considered comparatively negligible in the materials chosen for investigation. Subsequently, a change in the intercept was described briefly by Weltmann (*4*), and reference made to this paper for further information. This aspect of thixotropic breakdown will now be discussed.

Since structure produces yield value, it would be expected that breakdown would decrease the size of the yield value intercept. This does not seem to happen. The size of the intercept either remains practically unaltered or else actually increases. Stirring, however, does break down the initial static yield value, but this takes place so rapidly that no record of the breakdown is shown when the viscometer is hand-operated. The yield value actually appearing in the consistency curve is a dynamically determined one. The dynamic yield value is near the point where the velocity of structural breakdown is equal to the velocity of buildup. The static yield value is that which exists before the material has been touched by any stirring device. Obviously, it is greater than the dynamic yield value.

It has been found that for heavy pigment-vehicle suspensions, breakdown with time does not materially alter the yield value intercept (*5*). On the other hand, breakdown by rate of shear substantially increases it. In preventing a broken down thixotropic structure from reforming, there will be used a certain portion of the energy input for that purpose. This energy ($2\pi \times$ torque), not producing flow, must appear as an intercept on the torque axis. If breakdown is then increased by raising the power, a still larger amount of energy is required to maintain the new state, and, consequently, there will be a corresponding increase in the yield value intercept. Conditions are different when additional breakdown results from prolonging the application of a constant rate of shear. In this case there is no power increase and consequently there is no extension of the yield value intercept. Also, there is no decrease in the intercept because it evidently takes as much energy to maintain a given state of breakdown as it does to produce it.

DERIVATION OF COEFFICIENT OF THIXOTROPIC BREAKDOWN, V

Previously, equations were derived for the upcurve and for the loop area where the intercept was not affected by breakdown. Those equations were derived on the assumption that loss in torque is proportional to the increase in rate of shear. In this paper equations are developed where the intercept increases with thixotropic breakdown. In order to derive these new equations certain additional assumptions must now be made. They are: all downcurves intersect at a common point O, Figure 4. The upcurve also passes through this same point. Neither of these assumptions is strictly true; nevertheless, their adoption does not introduce serious errors in the final equations, as is shown below (in table, note the constancy of V).

The differential equation is set up as before, except that $(\omega - \omega_0)$ is substituted for ω (see Figure 4). The assumption is again made that the loss in torque (dT'), due to breakdown, is proportional to the velocity gradient at radius R. The instrumental constant, S, is equal to $(1/R_b^2 - 1/R_c^2)/4\pi h$, where h is the immersed height of the bob; R_b is the radius of the bob, and R_c the radius of the cup.

From similar triangles, Figure 4, it follows that

$$(dT' + dT)/d\omega = (T - T_{\omega_0})/(\omega - \omega_0) \qquad (27)$$

Using the same reasoning employed previously gives

$$2U/M = \ln k' + \ln[1/(\omega - \omega_0)^2] \qquad (28)$$

where k' is the constant resulting from integration and is equal to $(\omega - \omega_0)^2$ when U attains the hypothetical value of zero. Assuming a common crossing point, U becomes equal to $(T_{\omega_0} - T_2)S/\omega_0$ where T_2 equals f/C and C is an instrumental constant and equal to $S/\ln(R_c/R_b)$. This allows the substitution of $-(S/\omega_0)dT_2$ for dU. Differentiating Equation 28, making the substitution for dU, integrating, and condensing the constants gives

$$Jf = \ln k'' + \ln(\omega - \omega_0)^2 \qquad (29)$$

where J is equal to $2S/MC\omega_0$ and is the slope of the curve in Equation 32; k'' is a constant resulting from integration. If we let $2/MJ = V$, by differentiating and adding Equations 28 and 29, we get

$$V = -df/dU \quad (30)$$

or

$$V = (f_2 - f_1)/(U_1 - U_2) \quad (31)$$

V is the coefficient of thixotropic breakdown when the intercept increases with decreasing U. It is the increase in yield value per unit decrease in plastic viscosity. Its dimension is sec^{-1}.

The following equations also can be derived by the process similar to the one given in the previous work (*2*).

$$T = \frac{M(\omega - \omega_0)}{2S} \ln[k'/(\omega - \omega_0)^2] + T_{\omega_0} \quad (32)$$

$$A = M(\omega - \omega_0)^2/4S \quad (33)$$

Equations 28 and 32 can be combined with time in the same manner employed for Equations 15 and 17. Then k' is a function of time as k was shown to be in Equation 26.

It has been found experimentally that f is not affected by time when the top shearing rate or RPM is constant. Therefore, if f is plotted against t for different constant top RPM, a series of straight lines parallel to the t axis will result. This means that $f_1' - f_2' = 0$, $f_1'' - f_2'' = 0$, etc. Plotting f *vs.* $\ln(RPM - rpm_0)^2$ or against $\ln(\omega - \omega_0)^2$ for both t_1 and t_2 must give two linear coincident curves. These curves can be seen to be linear from Equation 32. They will be coincident because of the relation, $f_1' - f_2' = 0$, etc. The slope of the curve for t_1, then, will be the same as the slope of the curve for t_2, thus proving J and k'' to be independent of time. Because J and M are both independent of (Δt), V which is equal to $\frac{2}{MJ}$ is also independent of time.

EXPERIMENTAL RESULTS

The object of the following measurements is to show that Equations 28 and 29, and consequently Equation 31, are substantially in agreement with experiment, and therefore the assumptions previously made in regard to the crossing point are not too far out of line to be of practical importance.

From Equations 28 and 29, it is evident that U *vs.* $\ln[1/(\omega - \omega_0)^2]$ and f *vs.* $\ln(\omega - \omega_0)^2$ should be both linear relationships. This is shown to be a fact in Figures 5 and 6. Some of the data are given in Table I, which also contains the ratio $(f_2 - f_1)/(U_1 - U_2)$, demonstrating the constancy of V.

Table I. Data Taken from Figures 5 and 6 to Show Constancy of V

$V = (f_2 - f_1)/(U_1 - U_2)$. RPM = top rpm. U = plastic viscosity (poises). f = yield value (dynes/cm.^{2})

Pigment Suspension	rpm_0	RPM_1	U_1	f_1	RPM_2	U_2	f_2	V	Average Deviation of V %
1	60	200	583	17,400	300	415	24,000	39	
1	60	200	583	17,400	400	298	28,500	39	±0.9
1	60	300	415	24,000	400	298	28,500	38	
2	50	100	990	12,000	200	620	25,700	37	
2	50	100	990	12,000	300	465	32,000	38	
2	50	100	990	12,000	400	335	37,000	38	
2	50	200	620	25,700	300	465	32,000	41	±3.6
2	50	200	620	25,700	400	335	37,000	40	
2	50	300	465	32,000	400	335	37,000	38	
3	55	200	416	23,700	300	302	27,000	29	
3	55	200	416	23,700	400	215	29,400	28	±1.2
3	55	300	302	27,000	500	215	29,400	28	
4	25	100	250	7,400	200	186	8,700	20	
4	25	100	250	7,400	300	139	9,500	19	
4	25	100	250	7,400	400	115	10,000	19	±5.3
4	25	200	186	8,700	300	139	9,500	17	
4	25	200	186	8,700	400	115	10,000	18	
4	25	300	139	9,500	400	115	1,000	21	

In plotting Figures 5 and 6, it is immaterial, as far as $2/MJ$ or V is concerned, whether RPM or $(RPM - rpm_0)$ is used, for while the introduction of rpm_0 shifts the position of the curve, it does not affect the linearity or the value of $2/MJ$.

CONCLUSION

This paper, together with the previous work of the series (*2*), presents the fundamental principles of the thixotropic hysteresis loop, and the equations of thixotropic breakdown as produced by the rotational viscometer. It is hoped that this work will be found sufficiently complete to be useful to investigators who wish to study and measure thixotropic systems.

The practical aspect of the work depends upon the fact that industries that use materials of the pigment-vehicle type are often confronted with the problem of consistency changes arising from

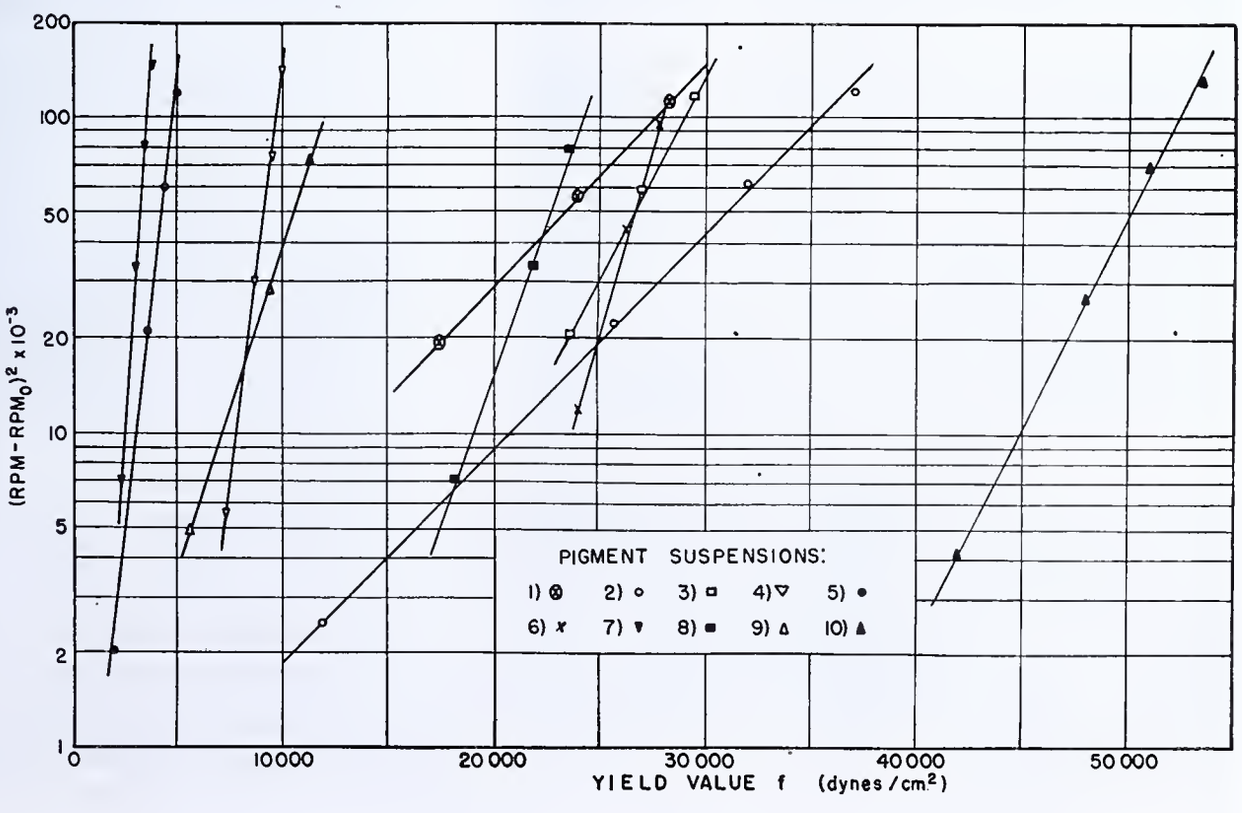

Figure 6. Experimental Data of $\ln(RPM - rpm_0)^2$ versus f for Various Pigment Suspensions

thixotropic breakdown. The plastic viscosity and yield value have one set of measurements when the substance is at rest; but these measurements change when the material is forced to flow. The magnitude of these new values will depend upon the applied rate of shear; and consequently can assume any of an infinite number of such values.

If this change to a new and unknown consistency affects the quality of the manufactured product, it is desirable to know beforehand how far thixotropic breakdown will go. If it continues too far, adjustments must be made in the original consistency, in the rate, or in the duration of shear applied to the material. The equations developed in this paper give the relationship between these factors and express them in the form of coefficients M, B, and V. They enable the investigator to calculate U and f for any rate of shear desired.

A well-known case where consistency changes can cause trouble is in the trapping of process printing inks. When printing is carried out at high rates of shear thixotropic changes can produce a reversal in the plastic viscosities of the inks. This in turn can cause a reversal in the order of trapping, so that a second-down ink, for instance, which should trap the third-down ink, is, instead, trapped by the third-down ink and pulled off the paper. Other difficulties can arise in the printing industry from thixotropic breakdown. An ink must not be too stiff to "follow the fountain". Thinning an ink down so that it can follow the fountain might result in trouble, if subsequent thixotropic breakdown reduced the plastic viscosity below that required for correct trapping. A better method might be to use an ink of suitable M, B, and f values.

Not only in printing but in any type of coating work—whether the process be carried out by means of a paint brush, a spray gun or a roller coating machine—problems are found involving thixotropic changes. These problems might not be recognized as such, but that is because general information on the subject is still very scanty. So far industry has made little commercial use of thixotropy; however, it is highly desirable that the industrial rheologist be thoroughly acquainted with thixotropic reactions, so that he understands the effect thixotropic breakdown has on consistency measurements. Without that understanding the rheology of plastic systems can become a confusing and contradictory subject. The equations given here should help the investigator meet that situation.

ACKNOWLEDGMENT

The authors are indebted to Interchemical Corporation for permission to publish this paper and to Miss Evelyn Berezin for assistance in carrying out the laboratory work.

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Measurement and Analysis of Small-Angle X-Ray Scattering

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Geiger counter methods can be used effectively for the recording of small-angle x-ray scattering. A graphical method of analyzing the data is presented and the results of such an analysis are given for one specimen each of gamma-alumina and carbon black.

THE scattering of x-rays at small angles is being used to study inhomogeneities in substances in the range of 10 to 200 Å. In a previous paper (*9*) the authors have given some qualitative results of a study of gamma-alumina, using photographic film to record the scattered radiation and a densitometer (*12*) to make the measurements. In a paper (*10*) presented at a meeting of the American Physical Society, it was shown that the Geiger counter could be used for studying this scattering. The present paper presents a simple method of graphical analysis of small-angle scattering data. The surprisingly satisfactory results of the method are demonstrated by its application to data obtained with a Geiger counter from gamma-alumina and carbon black.

APPARATUS AND TECHNIQUE

The complete apparatus employed in making the measurements is shown in Figure 1. The Philips small-angle apparatus is attached to a North American Philips Company standard x-ray diffraction unit and consists primarily of a 30-cm. collimator yielding a slit collimated beam. The slit dimensions are 0.3 × 3 mm. The specimen is mounted on a flat plate at right angles to the incident x-ray beam. The scanning unit is mounted on a track in such a manner that the distance from the specimen to the scanning slit can be readily changed. The entire scanning unit is fixed to the original track of the small-angle apparatus, so that it can be rotated about the line where the incident beam and the specimen intersect. The scattered radiation is scanned by turning a micrometer screw placed 60 cm. from the specimen, causing the scanning unit to rotate about the above axis. The movable track is suspended from two hardened steel pins that bear on V's cut in a yoke mounted on the Philips standard fixed track. A ball-bearing on the end of the micrometer screw rides along a V-groove cut in the movable track. The displacements are read on a scale and vernier that can be read to 0.05 mm., equivalent to about 0.35 minute of arc. The scanning unit contains a Geiger tube made by North American Philips and the circuits are essentially those described by Friedman (*7*). The combination of scaling circuits, impulse counter, and microammeter, and integrating circuits for the latter instrument easily allow measurements over an intensity range of 1 to 10,000.

Specimens are prepared by containing the material under examination in a circular opening in a thin metal sheet by the use of thin cellulose acetate membranes. The optimum specimen thickness for each type of sample handled is determined by setting the scanning unit at a fixed angle from the main beam and inserting specimens of varying thickness in the beam. By this method the specimen thickness producing the most intense scattering can be readily obtained.

Using the optimum specimen, the measurements are made by obtaining the number of counts per unit time interval at various angular displacements of the scanning unit from the main beam. The value obtained in this manner requires correction for two effects. The first is the background count of the apparatus which is obtained by daily calibration and is subtracted from all readings. The second correction is the subtraction of the air scattering caused by the main beam, diminished by the specimen absorption, passing through the air. The air scattering values are obtained for the correct beam strength by a method suggested by Campbell (*3*). The specimen is moved from its position at the collimator exit to a point between the x-ray tube window and the first slit of the collimator, so that all radiation, other than that scattered by the specimen at essentially zero angle is elimi-

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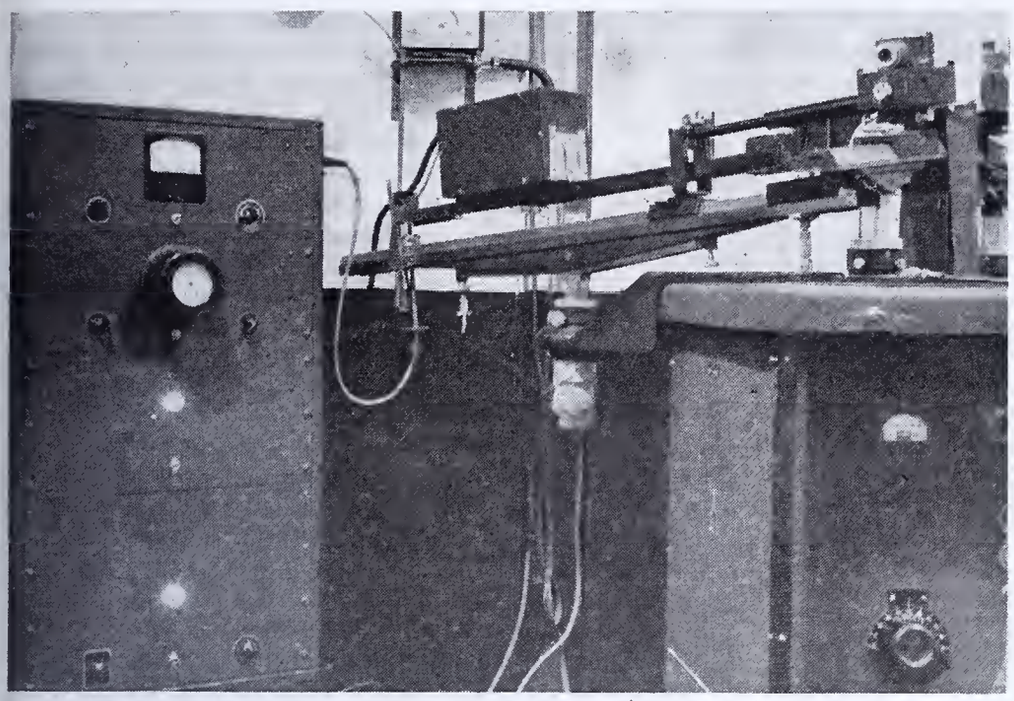

Figure 1. Diffraction Unit, Small-Angle Scatter Apparatus, Scanning Unit, and Geiger Counter

nated. Thus the air scattering can be measured directly and allowed for without the added complication of the small-angle scattering of the specimen.

THEORY

It has been shown (*2, 8, 11*) that the scattering of x-rays of wave length λ by a homogeneous assemblage of spherical particles of radius R may be given by the equation,

$$I(\theta) = CWR^3 \exp(-0.221\ k^2R^2) \qquad (1)$$

where $I(\theta)$ is the intensity of scattering as a function of θ, θ is half the scattering angle, k is $(4\pi \sin \theta)/\lambda$, W is the weight of specimen doing the scattering, and C is a constant for a given material. If there is a distribution of particle sizes given by $W(R)$, where $W(R)$ is the weight fraction of radius R, it can be shown that the curve of scattered radiation is then given by

$$I(\theta) = C\int_0^\infty W(R)R^3 \exp(-0.221\ k^2R^2)dR \qquad (2)$$

For the homogeneous case, Biscoe and Warren (*2*) suggest plotting $\log_e I(\theta)$ against k^2. (Equally satisfactory is a plot against $\sin^2\theta$, $\tan^2\theta$, or θ^2.) The more convenient plot (*9*) is one of $\log_{10} I(\theta)$ against r^2, where r is the distance in millimeters of the scanning slit from the center of the direct beam. When photographic recording was used r was a distance measured on the film. This logarithmic plot of Equation 1 yields a straight line whose slope is a single-valued function of R and whose intercept depends, among other things, on W and R^3.

When the assemblage contains a distribution of particle sizes, the logarithmic plot is no longer a straight line. Equation 2, in which $I(\theta)$ is experimentally determined and $W(R)$ is the desired function, suggests the use of Fourier transforms, and undoubtedly $W(R)$ can be calculated as the Fourier transform of some amenable analytical expression (or sum of expressions) for $I(\theta)$. In a recent note, Bauer (*1*) has discussed such an inversion, giving a method for transforming the observed small-angle scattering into a continuous distribution function. However, this method is somewhat involved and in many cases the data may not justify so detailed a treatment. The authors have been using a simple graphical method of approximating $W(R)$, not as a continuous function but rather as a set of discrete fractions. While such an interpretation and treatment of the scattering data are a distinct compromise, the results are, by empirical test, apparently useful and therefore this graphical method is here discussed.

EXPERIMENTAL RESULTS

As an example of this graphical method of analysis, the small-angle scattering from a heat-treated alumina gel was measured. Figure 2 is a plot of $\log_{10} I$ against r^2 in $(\text{mm.})^2$ obtained for this alumina using filtered copper radiation of $\lambda = 1.54$ Å. and a specimen-to-slit distance of 453 mm. Figure 2 also illustrates the method of graphical reduction.

A tangent to the experimental curve is drawn at the greatest angle of scattering studied. This tangent intersects the axis of ordinates at a value K_1. The values corresponding to this tangent are then subtracted from the original curve and a new corrected curve not containing the contribution of this fraction is obtained (shown in Figure 2 by the dashed line). In a similar manner the next tangent of minimum slope is drawn to the new curve with its intercept K_2. The procedure is repeated until the final points yield a straight line of intercept K_6. In this way, there are obtained six lines, with successively larger slopes, and inter-

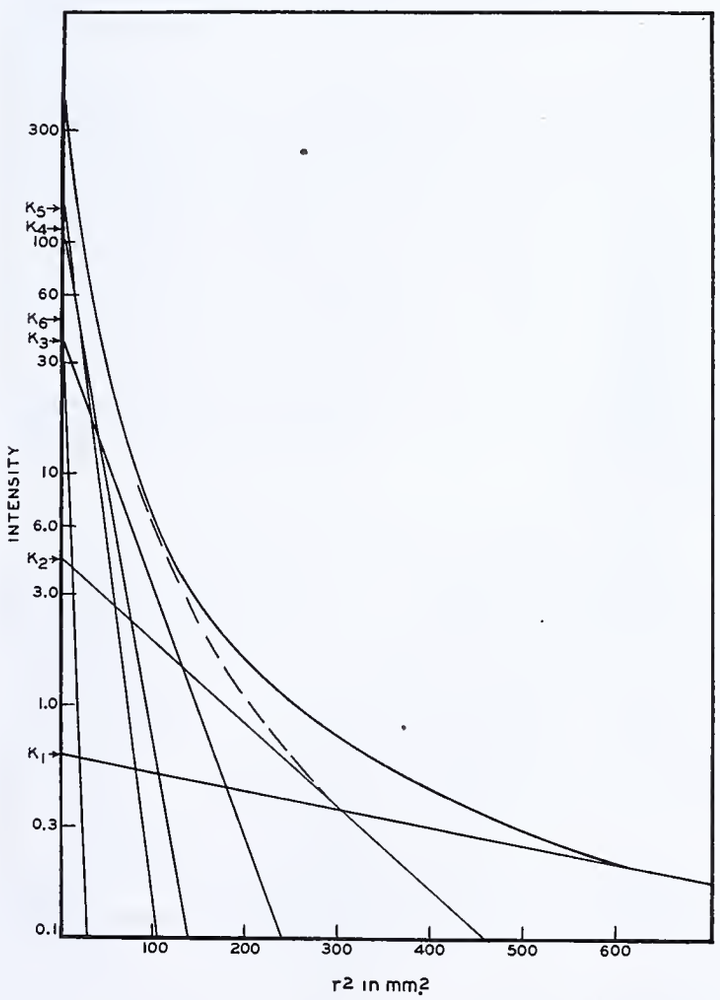

Figure 2. Plot of $\log_{10} I(\theta)$ against r^2 for Alumina Gel

Illustrating graphical breakdown into fractions

Table I. Calculation of Particle Sizes from Small-Angle Scatter Data

	Ordinate Intercept	Calculation of Slope $\Delta \log I$	Δr^2	$\Delta \log I/\Delta r^2$	R	R^3	$K/R^3 \times 10^4$	Weight Proportion
K_1	0.61	0.484	630	0.000769	10	1,000	6.10	0.194
K_2	4.3	1.633	457	0.00358	22	10,600	4.06	0.129
K_3	39	2.591	240	0.0108	37	50,600	7.71	0.245
K_4	115	3.061	140	0.0219	54	157,000	7.32	0.232
K_5	140	3.146	107	0.0294	62	238,000	5.88	0.186
K_6	48	2.681	33	0.0812	103	1,092,000	0.44	0.014
						Total	31.51	1.000

Calculated surface = 350 sq. meters per gram.

cepts $K_1 \ldots K_6$. The slopes of these lines are calculated, the corresponding radii are read from an appropriate graph of slope against R on log-log paper (see *9*, Figure 3), and the weight fractions of particles of each of the radii can then be calculated. The complete calculation is outlined in Table I. This tabulation indicates the values of the intercept K's which have been shown to be proportional to $R^3W(R)$, the calculation of the slopes of the various lines, the corresponding radii, and the cubes of these values. When each K is divided by its appropriate R^3 the resulting values are proportional to the weight fraction of that size particle.

From the weights of the fractions one can compute the surface area of the specimen material. Calculations using an average particle size have been made by Elkin, Schull, and Roess (*4*). In their own studies the authors have found that for gel-based catalysts of various activities an acceptable correlation can be obtained between the surface area calculated from these fractions and catalytic activity.

It is of some interest to demonstrate how closely the graphical reduction of the experimental curve into a sum of a set of straight lines represents the original curve. This calculation has been performed and the results are given in Table II, where the values of I for certain values of r^2 are presented along with the values at the same r^2 obtained by analytically summing the straight lines.

The deviations from the experimental values are seen to be largest at the highest intensities where the curve is rising almost vertically, and hence represent the values most difficult to read from the graph.

Another application of this graphical method can be seen in the analysis of a scatter curve obtained from a sample of carbon black obtained from J. A. Hillier of the RCA Research Laboratories and thought to consist of nearly spherical uniform particles on the basis of many electron microscope pictures. Figure 3 shows the scatter curve (run at a specimen-slit distance of 235 mm.) obtained from this material. It is clear from a comparison of the shapes of the curves shown in Figures 2 and 3 that the carbon must consist of larger particles than the alumina gel. The results of the graphical analysis bear this out (Table III).

The calculated surface area of this material is 280 square meters per gram and that determined by nitrogen absorption is 450 square meters per gram. This may be compared with the surface area of the alumina gel which was calculated to be 350 square meters per gram. The actual measurement of the small-angle scatter yields a curve of scattered intensities as a function of θ and it is this curve that we analyze and convert into a particle distribution. The result can be very misleading if the total observable scattering should arise from only a small proportion of the sample. Thus, if the samples used in these analyses were heat-treated more severely, the particle distribution would shift as larger sized particles were formed. When most of these particles reached a radius of well over 100 Å. the largest part of the scattering would be very close to the main beam, but a minor amount of scatter due to the smaller particles would occur at reasonable angles and might conceivably be analyzed as due to the entire sample. This can easily be seen in Table IV, taken from a paper by Fankuchen and Mark (*6*), which shows the angles at which the scattering falls to half its value at $\theta = 0$ for various values of R.

The only evidence of this effect would be a lesser intensity at the larger angles and sometimes an increase at the lowest scattering angles reached by the authors' experimental technique. This effect has been observed (*9*) by the authors on gamma-alumina. There is naturally no foolproof guide to these effects other than experience and knowledge of the past history of the sample. Certain of these effects can even be duplicated by variations in specimen thickness and intensity of the original beam. Conse-

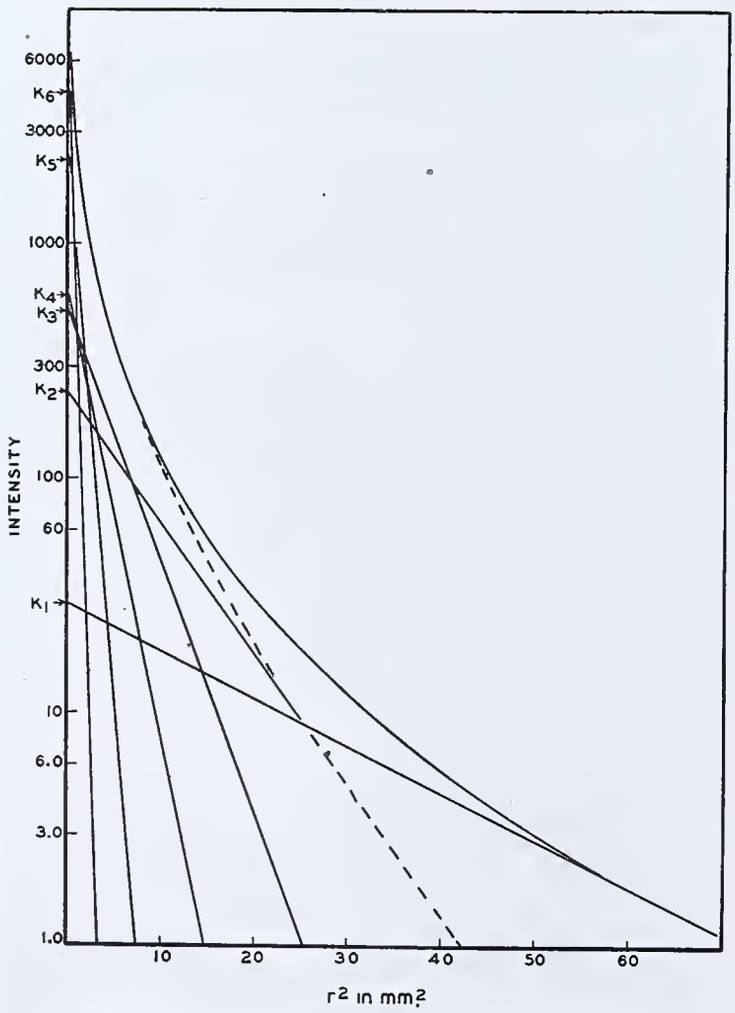

Figure 3. Plot of $\log_{10} I(\theta)$ against r^2 for Carbon Black

Illustrating graphical breakdown into fractions

Table II. Comparison of Experimental and Calculated Small-Angle Curves

r^2	I (Experimental)	I (Calculated)
5	280	245
25	80	83
50	26.5	28.8
100	6.4	6.7
150	2.75	2.71
200	1.60	1.54
300	0.74	0.74
400	0.44	0.46

Table III. Weight Distribution of Particles in Carbon Black

Radius, Å.	Weight %
29	14.7
46	29.4
63	25.7
85	12.2
130	12.1
210	5.9

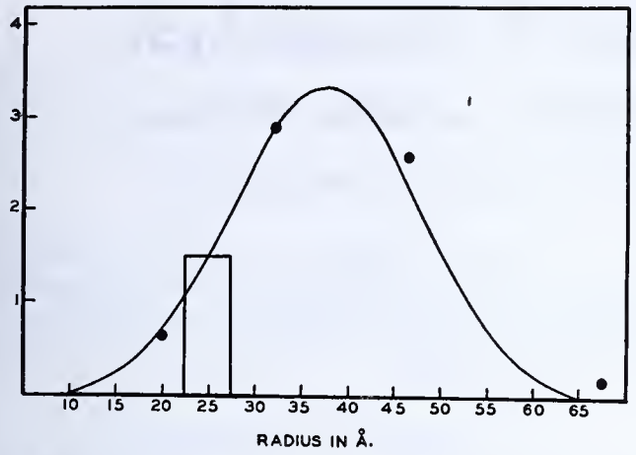

Figure 4. Assumed Distribution Curve Used in Calculation of Synthetic Small-Angle Scatter Plot

Points spotted are results of graphical analysis

quently, this graphical method of determining particle size distribution from small-angle scatter may be a useful tool when the limitations and range of failure of the method are recognized.

Having used the graphical method to evaluate two experimental curves, it may be informative to utilize the method on a synthetic curve obtained from an assumed particle distribution. This has been carried out in the following manner.

Figure 4 shows an assumed continuous distribution curve with the area under the curve equal to 100% of the material. The curve was then approximated by a series of twelve fractions taken at 5 Å. intervals from 10 to 65 Å.—that is, for the continuous curve there was substituted a series of rectangles of width 5 Å. and height such that the area of each rectangle equaled the area under the corresponding part of the continuous curve. Thus the rectangular block in Figure 4 would represent the 25 Å. particles, and would include the range from 22.5 to 27.5 Å. From these relative weights and the corresponding radii, the slope and a number proportional to the intercept were obtained. These data were then used to draw a series of straight lines of correct slope and ordinate intercept such that their sum constituted a curved line which represented the small-angle scatter of a sample containing such an original particle distribution. This curve was broken down in turn by the usual graphical method and yielded the results given in Table V.

Table IV

Radius, Å.	Angle 2 θ at Which I Has Fallen to Half of Initial Value, Min.
25	63.0
50	31.5
100	15.8
200	7.8
400	3.9

When these data are spotted in Figure 4, multiplied by a constant to place them in the same range as the original distribution curve, it can be seen that the breakdown of a continuous curve can yield, via this method of discontinuous evaluation, a fair approximation of the original sample. Further refinements of this method have been attempted and evaluated by drawing a continuous cumulative percentage curve and then differentiating at as many points as desirable to yield a continuous distribution function. No improvement is noted, because of the differences possible in the exact location of the cumulative curve and the arbitrary manner in which the inflection point must be chosen. Thus, we conclude that the "shot distribution" is as useful a set of values as any other that may be obtained by the graphical breakdown.

Some of the difficulties can probably be overcome by carrying out the measurements under standardized conditions such that intensities from different specimens of similarly constituted materials can be compared with one another. This will require careful control of the constancy of the x-ray beam intensity and uniformity in specimen preparation. It would then be meaningful to compare intensities from different specimens and thus by suitable calibration tell what proportion of the specimen is observable by the small-angle technique used.

CONCLUSION

At this stage of development it would be premature to take too seriously these computations of particle size distribution. However, whenever relative surfaces have been calculated based on these studies and when comparisons between these surfaces and properties based on them, such as catalytic activity, are made there has always been a reasonably good correlation.

Undoubtedly the small-angle scatter reflects an inhomogeneity in the specimen. This inhomogeneity is of the same order of magnitude as what we here like to call particle size. Perhaps in many cases, further study may show that particles in the sense in which we now think of them do not exist; the small-angle scattering merely reflecting this inhomogeneity in structure which is responsible in large part for the physical behavior of the specimen. Should this possibility be shown to exist, there nevertheless may still be a justification for continuing to talk of particles—the justification being that it works.

Table V. Analysis of Synthetic Scattering Curve

Radius, Å.	Weight %
20	10
32	46.5
46.5	41.0
67.5	2.5

There seem to be considerable possibilities for improvement experimentally. Thus, measurements at the very small and large angles at present offer some difficulties, but the authors feel that they can be made satisfactorily. At very small angles (even to 0°) the two-crystal method (*5*) used in conjunction with a Geiger counter with the sensitivity of the equipment described above should enable these measurements to be carried down to almost any angle. At intermediate angles there seems to be no serious difficulty, but at large angles reliable measurements are difficult because the intensity is low. However, at this point the change in intensity with angle is usually small and consequently the width of the scanning slit may be increased to yield a larger number of counts.

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Taken in part from a thesis submitted by M. H. Jellinek in partial fulfillment of the requirements for the degree of doctor of philosophy in chemistry at The Polytechnic Institute.

Polarographic Determination of Acetaldehyde

Application to Routine Determination in Complex Mixtures

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A polarographic study has been made of acetaldehyde, and a method is described for the polarographic determination of acetaldehyde in the complex liquid mixtures and in liquefied C_4 distillation fractions obtained in the conversion of ethanol to butadiene. The method is simple, rapid, and accurate, and is superior to the titrimetric methods which are usually used.

IN CONNECTION with research and production control on the single-step process for the conversion of ethanol to butadiene it became necessary to have a rapid, simple, and accurate method for the determination of acetaldehyde. It was desired to determine acetaldehyde in the concentration range from 0.1 to 10% in alcohol denatured with varying amounts of acetaldehyde and in complex liquid mixtures which might contain C_3, C_4, C_5, and C_6 olefins and diolefins, C_2 and C_4 alcohols, diethyl ether, water, and small amounts of unknown organic compounds. It was also desired to apply the same method to the determination of acetaldehyde present in the liquefied C_4 samples obtained by the fractionation of butadiene-containing samples. The presence of acetaldehyde is due to the formation of azeotropes of it with butadiene and various butenes, and to difficulties in the separation of the C_4 fraction from other azeotropes containing acetaldehyde.

Investigation of the methods generally used for the determination of acetaldehyde showed the titrimetric hydroxylamine method (*2*, *3*) as modified to be the most accurate and reliable, although it was not entirely satisfactory. This was also found true by Russian workers (*7*, *8*) dealing with the same problem. While the hydroxylamine method could be readily applied to samples containing only small amounts of components boiling below room temperature, the titration was difficult to carry out because of the effect of the ethanol and other solvents present on the indicator. The substitution of an electrometric detection of the end point and the use of synthetic mixtures duplicating end-point conditions were unsatisfactory. The determination of acetaldehyde by the hydroxylamine method in samples containing considerable amounts of volatile material as in samples containing over 5% acetaldehyde or C_4's, or in C_4 fractionation samples, was time-consuming and tedious, owing to the necessity of carrying out the determination as a sealed tube reaction.

The difficulties attending the use of the hydroxylamine method led to a study of the polarographic method and a satisfactory solution was found. The procedure described is applicable to the complex mixtures previously indicated which were found to contain no detectable amounts of any other compound reducible at less negative potentials at the dropping mercury cathode (Figure 1). In the presence of substances reducible at different voltages from acetaldehyde the method could be applied with slight modification. In connection with this method a study was made of the polarographic behavior of acetaldehyde at various temperatures, hydrogen-ion concentrations, and acetaldehyde concentrations in order to make more extensive use of the polarographic method.

The polarographic behavior of acetaldehyde has been discussed by several workers whose results are summarized by Kolthoff and Lingane (*6*). Shikata and his co-workers (*9*, *10*) noted the polarographic determination of minute amounts of aldehydes including acetaldehyde in alcoholic beverages. The samples were dissolved in aqueous electrolyte solution, freed from dissolved oxygen by having hydrogen bubbled through them, and examined polarographically. The wave at decomposition potential −1.6 volts was assumed to be due to acetaldehyde for 10^{-4} *M* concentration. Smöler (*11*) studied the mechanism of polarographic reduction of acetaldehyde and described its determination in fermentation products; unfortunately, the results are not very conclusive.

APPARATUS AND MATERIALS

A Leeds & Northrup Electro-Chemograph and a Fisher Scientific Company Elecdropode were used for the polarographic measurements. For the constant-temperature experiments, water from a thermostat was circulated through a jacket surrounding the polarographic cell which kept the temperature in the cell constant to ±0.1° C. The drop time of the capillaries used for the analytical studies could be controlled to between 3 and 5 seconds by regulation of the mercury column. It was unnecessary to calibrate the apparatus, since variations in conditions are accounted for by use of a standard sample whenever a series of unknown samples is analyzed. If the instrument is in continuous use for several hours, a standard sample should be analyzed each hour or two as described below.

The acetaldehyde used for standardization and the preparation of synthetic mixtures was C.P. grade which was purified before use by fractionation through an efficient distilling column containing Stedman packing. Its physical constants checked those reported in the literature. The other chemicals used were of C.P. grade. The base solution used in the analytical procedure was 0.1 *M* lithium chloride. The following buffer solutions were prepared: (1) the buffer of pH 6.8 was 0.1 *M* in lithium, 0.05 *M*

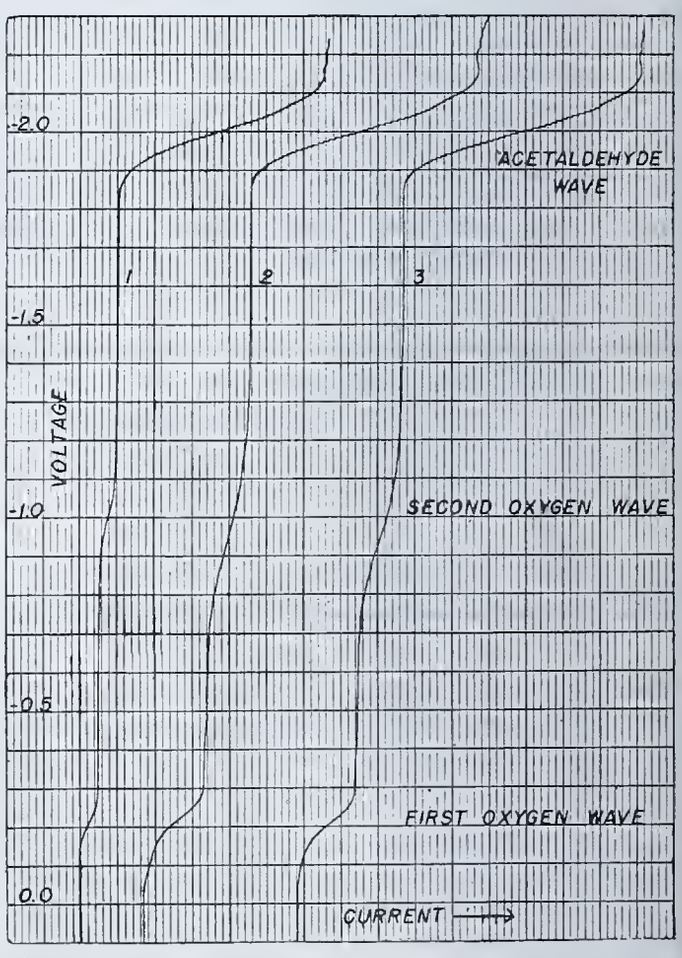

Figure 1. Current Voltage Curves (Electro-Chemograph) for Butadiene Condensates

Temperature, 25° C. Supporting electrolyte, 0.1 *N* LiCl
Concentration of condensate, 1/400
Sensitivities: (1) 1/400, (2) 1/40, (3) 1/40

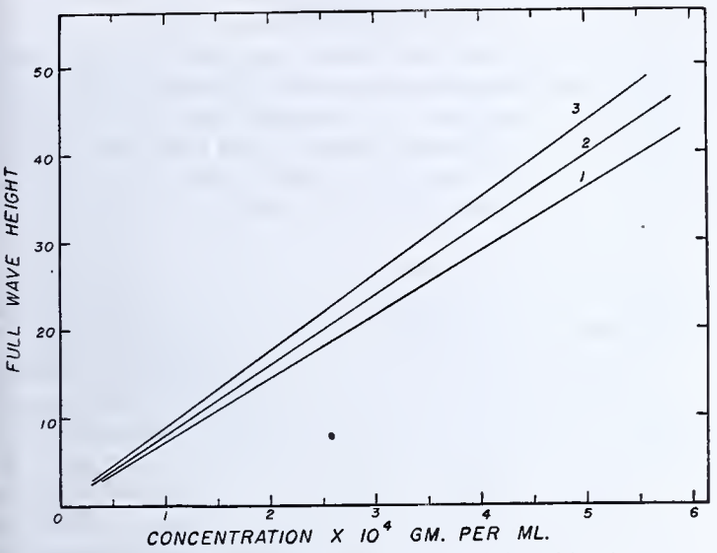

Figure 2. Effect of Temperature on Wave Height

Sensitivity calculated to 1/50. pH, 6.8
Temperature: (1) 20° C., (2) 25° C., (3) 30° C.

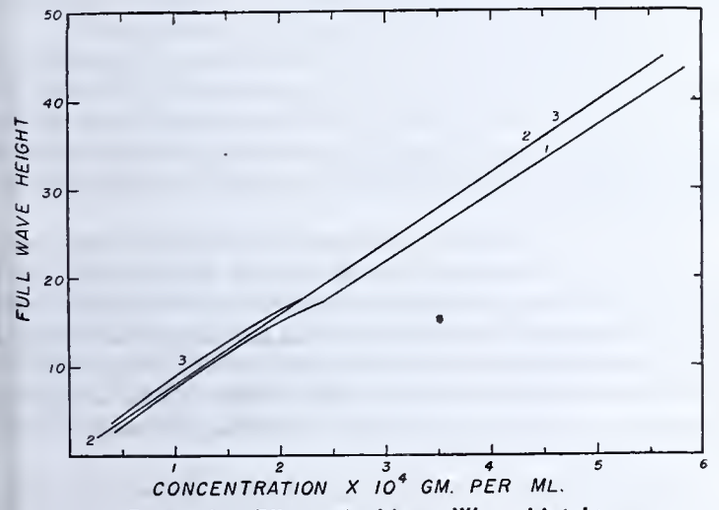

Figure 3. Effect of pH on Wave Height

Sensitivity calculated to 1/50. Temperature, 25° C.
pH: (1) 8.8, (2) 6.8, (3) 12.7

in acetate, and 0.05 M in chloride ion, and had enough acetic acid added to adjust the pH to 6.8; (2) the buffer of pH 8.8 was prepared by adjusting the pH of 0.2 M lithium hydroxide with acetic acid; (3) the solution of pH 12.7 was 0.11 M in lithium, 0.06 M in hydroxyl, and 0.05 M in chloride ion. For the study of the factors affecting the polarographic determination of acetaldehyde, ten solutions were prepared by diluting weighed samples of acetaldehyde with distilled water; these solutions had the following concentrations in grams per ml. $\times 10^{-3}$ (molar concentration in parentheses): 5.26 (0.120), 4.86 (0.110), 3.96 (0.090), 3.61 (0.082), 3.11 (0.071), 2.47 (0.056), 2.12 (0.048), 1.62 (0.037), 0.96 (0.022), 0.48 (0.011). These solutions were diluted 1 to 10 with appropriate base solutions before use. Two solutions for standardization were prepared by dissolving weighed amounts of acetaldehyde in 0.1 M lithium chloride solution; one solution contained 5.0×10^{-5} gram of acetaldehyde per ml. (0.00114 M) and the other contained 5.0×10^{-4} (0.0114 M). The latter two solutions were found to be stable for at least 3 weeks. The solution used depended on the range of concentration of the samples being analyzed.

STUDY OF TEMPERATURE, pH, AND CONCENTRATION EFFECTS

The capillary used in these studies had a drop time of 3.76 seconds per drop for a mercury column 51.3 cm. high. The weight of mercury dropped per second was 1.093 mg. The value of $m^{2/3}t^{1/6}$ is, accordingly, 1.32 mg.$^{2/3}$ sec$^{-1/2}$.

Since the effects of temperature and pH on the diffusion current are the most important factors in the polarographic determination of organic compounds, the effect of varying these conditions was studied. Polarograms were obtained at 25° C. for pH values of 6.8, 8.8, and 12.7; at pH 6.8 for temperatures of 20°, 25°, and 30° C. All wave heights were recalculated to a sensitivity of 1/50 for the Elecdropode. Half-wave potentials were referred to the saturated calomel electrode.

Diffusion Current. The temperature coefficient for the diffusion current, $(1/i)(di/dT)$, at pH 6.8 was found to be approximately 1.8% per ° C. over the range of 20° to 30° C. (Figure 2). This is in agreement with normal behavior, as the diffusion currents for most ions and molecules have temperature coefficients of 1.5 to 2.0% per ° C. (5).

The concentration–diffusion current curve at pH 6.8 showed a linear relation over the whole concentration range studied, while the curve at pH 8.8 showed a linear relation only over certain concentration ranges (Figure 3). The curve at pH 12.7 showed a linear relation between 2.5 and 5.5 $\times 10^{-4}$ gram per ml. (0.0057 to 0.0125 M). In this range the curve is identical with the curve for pH 6 8. For determining low concentrations of acetaldehyde it is best to use a base solution of high pH between 12 and 13, since the curve at pH 12.7 is linear for low concentrations in limited regions and has a large slope. It was found impractical to use a base solution of pH less than 6.8, as the interference from the hydrogen-ion wave is appreciable below this pH.

A linear relation was found between the concentration and the reading at −2.14 volts (dropping mercury electrode *vs.* pool) (Figure 4). The linearity of this relation was of the same order as that obtained for the full wave heights.

Half-Wave Potential. The effect of temperature, pH, and concentration on the half-wave potential was also observed. All half-wave measurements were made on the Elecdropode and were referred to the saturated calomel electrode. The half-wave potential is independent of temperature at pH 6.8 in the range of 20° to 30° C. with a shift of less than 0.01 volt, which is not significant as the limit of sensitivity of the instrument is about 0.005 volt. With 0.0119 M acetaldehyde solution (5.26×10^{-4} gram per ml.) potentials at pH 6.8, 8.8, and 12.7 were −1.89, −2.04, and −1.89 volts *vs.* the saturated calomel electrode at 25° C. A change in half-wave potential with concentration was noted for the concentration range studied (0.00114 to 0.0114 M acetaldehyde). The shift for tenfold change in concentration is about 0.08 volt at pH 6.8 and 25° C.; similar results were obtained using other buffer solutions.

ANALYTICAL PROCEDURE

The method is based upon the fact that the diffusion current of acetaldehyde is a linear function of concentration under the conditions used. The wave heights, galvanometer deflections, or similar measures of the diffusion currents are determined at a potential difference between the cell mercury pool and the dropping mercury electrode of −2.14 volts for the unknown samples, a standard sample containing a known amount of acetaldehyde, and the sample-free base solution. The concentration of acetaldehyde in the unknown sample is readily ascertained by a simple proportion involving the concentration of the standard sample. The removal of oxygen from the base and sample solutions is unnecessary, since the effect of oxygen is compensated for

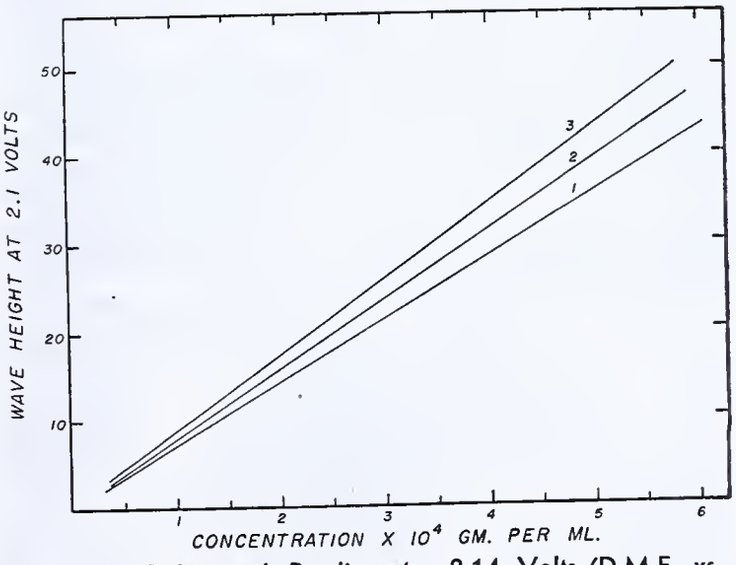

Figure 4. Polarograph Reading at −2.14 Volts (D.M.E. *vs.*, Pool)

Sensitivity calculated to 1/50. pH, 6.8
Temperature: (1) 20° C., (2) 25° C., (3) 30° C.

Table I. Determination of Acetaldehyde in Butadiene Condensate

Sample No.	Acetaldehyde Found: Using 0.1 N LiCl, Using full wave height	Using 0.1 N LiCl, Using reading at −2.14 volts	Using a Buffer of pH 6.8, Using full wave height	Using a Buffer of pH 6.8, Using reading at −2.14 volts
	%	%	%	%
5970	1.70	1.71	1.75	1.75
5486	1.61	1.60	1.64	1.65
5492	1.34	1.34	1.29	1.30

Table II. Determination of Acetaldehyde in Known Mixtures

Calcd. concn., mg./ml.	0.088	0.122	1.22
Polarographic detn., mg./ml.	0.091	0.120	1.24

in the measurement of the base solution. Polarograms of acetaldehyde and of typical samples containing acetaldehyde indicated that the use of a maxima suppressor was unnecessary.

A sample weighing 0.10 to 0.25 gram, depending on the concentration of acetaldehyde present, is taken in a sealed glass ampoule (*4*). If volatile compounds are present, the ampoule is immersed in an acetone–solid carbon dioxide or similar cooling bath for filling and sealing. The ampoule and several glass marbles or 2.5-cm. (1-inch) lengths of 7-mm. glass rod are introduced into a 400-ml. (16-ounce) citrate of magnesia bottle containing 50 ml. of base solution (300 ml. should be used for very volatile samples such as liquid C_4 hydrocarbon fractions). The bottle is stoppered and the ampoule is crushed by shaking the bottle, thus diluting the sample to 50 or 300 ml. A portion of the solution is transferred to the polarographic cell and a reading is taken of the current at a potential difference between the cell mercury pool and the dropping mercury electrode of −2.14 volts. A reading at this potential difference is on the plateau of the polarographic wave due to acetaldehyde (Figure 1). The wave height due to the reduction of acetaldehyde is calculated by subtracting the galvanometer reading of the base solution from that for the sample solution. The wave height is then compared with that obtained with a known amount of acetaldehyde in order to determine the acetaldehyde in the unknown, the concentrations being directly proportional to the wave heights.

It was thought necessary to show that no difference in results is obtained when samples of the liquid condensate obtained in the butadiene process are analyzed in 0.1 M lithium chloride whose pH is 6.8 instead of in a buffer solution of the same pH. Three samples of condensate were analyzed in a buffer of pH 6.8 and in 0.1 M lithium chloride; the results agreed well within the limit of error of the method (Table I).

ANALYTICAL RESULTS

The results obtained with known mixtures containing acetaldehyde confirmed the linearity of the diffusion current–concentration relationship and indicated the satisfactory order of accuracy and precision of the polarographic method. A few typical results are shown in Table II.

In Table III are given the results obtained by the polarographic method described and by the titrimetric hydroxylamine method for the determination of acetaldehyde in multicomponent mixtures obtained in the catalytic conversion of ethanol to butadiene. These mixtures contained considerable amounts of ethanol, diethyl ether, and water; and smaller amounts of C_4 and C_6 unsaturated hydrocarbons, butanol, and other organic compounds; no aldehydes other than acetaldehyde were present in detectable amounts. A considerable number of synthetic samples consisting of liquefied butadiene and butenes, and acetaldehyde were prepared and analyzed; the results are shown in Table IV. The analysis of similar mixtures for acetaldehyde by the hydroxylamine method showed a lower order of accuracy and precision with occasional errors of 50 relative %. Subsequently a new set of butadiene–2-butene–acetaldehyde mixtures was prepared and analyzed; the results are also shown in Table IV.

DISCUSSION

The best conditions under which acetaldehyde can be determined polarographically are governed by the concentration range in which one is working. For the range of 0.50×10^{-4} to 5.0×10^{-4} gram per ml. (0.0014 to 0.0114 M) a 0.1 M lithium chloride solution or a buffer solution of pH 6.8, if necessary, is most suitable. For lower concentrations a 0.1 M lithium hydroxide solution is most suitable.

The use of the increment method for the determination of acetaldehyde is inadvisable because of the shift in half-wave potential over the concentration range covered.

If substances are present which are more readily electroreducible than acetaldehyde, such as formaldehyde or unsaturated aldehydes, it is necessary to make an additional measurement of the diffusion current in the neighborhood of −1.8 volts and to use the difference in diffusion currents to calculate the necessary wave heights; however, in such cases it is best to obtain the complete curve using the Electro-Chemograph, since in some cases the reduction of one substance affects the subsequent reduction of another (*1*). If there are no interfering substances, the Elecdropode at a setting of −2.14 volts is preferred with sensitivities in the range of one fifth to one fiftieth. A sample of unknown nature should be analyzed by means of the complete curve.

Error would be caused by the presence of other substances electroreducible by the dropping mercury electrode in the same potential range as acetaldehyde. Since the sample size is usually less than 0.3 ml., only a negligible error is introduced by neglecting the change in volume when the sample is diulted with the base solution.

In the case of liquefied C_4 samples, the method has been found satisfactory for concentrations up to 15% acetaldehyde. The error in the case of C_4 samples is believed to be due to the difficulty of sampling a liquefied gas and to the degasifying action of the C_4 hydrocarbons upon the oxygen content of the solution, resulting in different oxygen contents for the sample, and for the standard and base solutions with a resulting inaccuracy in the wave height of the sample.

The polarographic method is very rapid. While a single determination takes about 20 minutes, a group of determinations takes about 12 minutes per sample plus 10 minutes for running a standard sample and the base solution. These periods of time include sampling and the necessary weighings. The method has been in constant use for over 20 months and over 4000 determinations have been performed on many types of samples in addition to those described. The method has been used successfully by laboratory technicians after several hours of train-

Table III. Determination of Acetaldehyde in Complex Mixtures

Sample No.	Acetaldehyde: Polarographic method	Acetaldehyde: Hydroxylamine method
	%	%
5967	1.03, 1.01, 1.00	1.0, 1.0
5982	0.97, 0.97	1.0, 0.9
5987	0.85, 0.86	0.9, 0.9
6507	1.12, 1.10	1.3

Table IV. Polarographic Determination of Acetaldehyde in Synthetic Mixtures of Liquefied C_4 Hydrocarbons

Acetaldehyde taken, %	0.58	0.71	0.92	1.00	1.63	2.09	3.39	5.3	6.5	8.7	10.4	13.1
Acetaldehyde found, %	0.50	0.76	1.10	0.98	1.63	2.29	3.42	5.0	6.6	8.7	9.8	13.4
	0.50	0.82	1.05	1.02	1.52	2.22	3.48	4.7	6.3	8.4	9.6	12.7
	0.52											

Butadiene–2-butene–acetaldehyde mixtures

Acetaldehyde taken, %	0.67	1.25	4.26	9.34	19.0
Acetaldehyde found, %	0.69	1.16	4.23	9.23	19.0

g in sampling and in adjusting the manually operated Elec-ropode. An experienced technician was able to analyze 30 search samples in 5 hours.

As compared to the hydroxylamine method which was found be the best of the ordinary titrimetric methods, the polaro-raphic method has the following advantages. It is very much ster; the hydroxylamine method requires about 60 minutes for ie determination of acetaldehyde in a liquefied C_4 sample com-ared to 12 to 20 minutes for the polarographic method. It is mpler to use and can be readily taught to technicians and chem-ts. For the type of samples discussed it is more accurate; his fact is of special importance for samples of low acetaldehyde oncentration. The minute amount of sample necessary for the olarographic method is of great advantage in research work. he only apparent disadvantage is the cost of the polarograph, hich is rapidly recovered in saving of manpower if a consider-ble number of samples are analyzed.

A pilot-ion technique for the polarographic determination of cetaldehyde is being investigated.

ACKNOWLEDGMENT

The authors wish to acknowledge the assistance of Benj. Warshowsky and Joyce Becker with the experimental work.

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Determination of Beryllium in Ores

Fluorometric Method

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A fluorometric method using quinizarin in an alkaline citrate solution has been developed for the determination of beryllium in ores. Preparation of the sample for analysis consists essentially of fusion with sodium carbonate and borax glass, solution of the melt in hydrochloric acid, and proper dilution. Directions are given for removal of other ions when necessary. A simple visual comparator or a photoelectric instrument may be used for the measurements. The effect of changes in dye concentration and beryllium content on the characteristics of the fluorescence, the influence and removal of other ions, and the effect of light, temperature, and time are discussed. The method is particularly practicable for routine analysis since reliable results may be obtained with rapidity and ease.

THE fluorometric method described herein was developed in answer to the need for a rapid, reliable method which could e used for large numbers of beryllium determinations on both ow- and high-grade materials. It has been successfully used for he past 18 months in the analysis of hundreds of samples.

Fluorescent reagents were first used in 1933 when Zermatten 7) found that morin reacted with beryllium in the presence of lkali to produce a yellow-green fluorescence and described a pot test based on the reaction. Sandell (*4*, *5*) made a thorough tudy of the morin reaction for beryllium and its application to he determination of beryllium in silicates.

White and Lowe (*6*) described 1-amino-4-hydroxyanthraquinone as a less sensitive but more specific reagent than morin or beryllium. Palmes and Alford (*3*) adapted White and Lowe's ualitative method to the quantitative determination of minute mounts of beryllium in biological materials, and also proposed he use of quinizarin (1-4-dihydroxyanthraquinone), a similar eagent.

Tests of the three reagents (morin, 1-amino-4-hydroxyanthraquinone, and quinizarin) in this laboratory showed quinizarin to e the most suitable. The method devised measures the amount f beryllium present by comparison of the intensity of the luorescence of the unknowns with that of a set of similarly pre-ared standards. The fluorescence is produced when an alkaline olution containing beryllium and quinizarin is exposed to ultra-iolet light. The comparison may be made either directly in a imple visual comparator, or alternatively with a photoelectric luorometer; both give about the same accuracy.

A single complete analysis can be made in about 2 hours on amples free of interfering ions; ten to fifteen determinations can be made in an 8-hour day. Samples which contain interfering ions require 1.5 to 2 days for a single determination, but even under these conditions fifteen to twenty analyses can be run in 4 days.

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REAGENTS AND APPARATUS

All reagents were C.P. chemicals with the exception of the quinizarin, aerosol, and the borax glass used in the flux. All reagents were tested for fluorescence before they were used.

Fusion mixture, 3 parts of anhydrous sodium carbonate with 1 part of borax glass (technical grade such as that used in fire assaying).

Quinizarin (1,4-dihydroxyanthraquinone). Technical-grade quinizarin was boiled with decolorizing carbon in acetone, filtered, and crystallized. The dye was then recrystallized from acetone and air-dried. Stock solution, 0.29% in C.P. acetone. Working solution, stock solution diluted (1 to 10) with C.P. acetone. These solutions were stored in glass-stoppered bottles.

Sodium citrate, 5%.

Sodium hydroxide, 1, 2, 5, 10, and 20%.

Laboratory aerosol, the commercial 10% product diluted (1 to 10).

Hydrofluoric acid, 48%.

Hydrochloric acid, 10 and 30% by volume.

Ammonium hydroxide, concentrated, and a 0.1% (by volume) wash solution.

Filter paper pulp, prepared from Whatman No. 41 H in a Waring mixer.

Standard Beryllium Solutions. A solution of beryllium chloride was prepared in the following manner. C.P. beryllium nitrate in solution was treated with 8-hydroxyquinoline to remove traces of aluminum and iron, and excess 8-hydroxyquinoline was destroyed with nitric and sulfuric acids. Beryllium hydroxide was precipitated with ammonia. The precipitate was dissolved in 10% hydrochloric acid, and diluted until 1 ml. was equal to approximately 10 mg. of beryllium oxide. The solution was

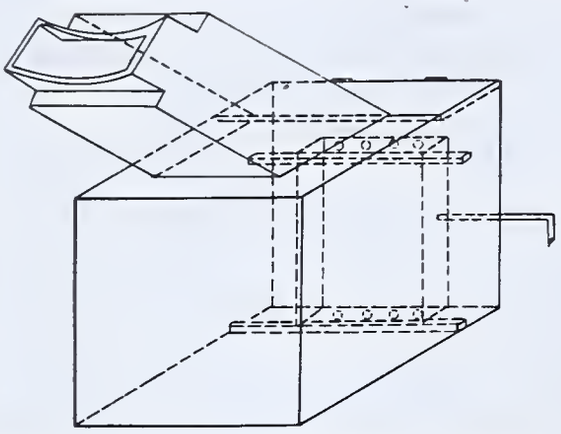

Figure 1. Front View of Fluorometric Comparator

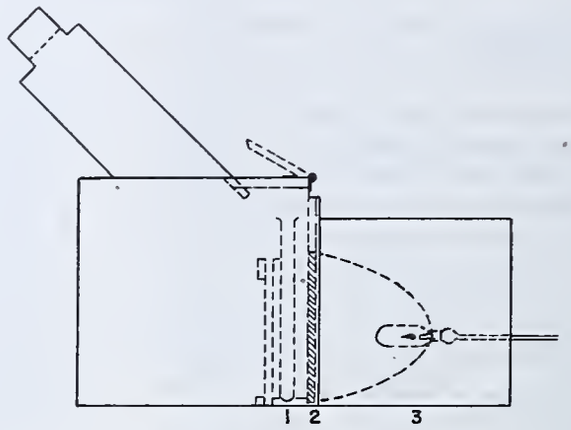

Figure 2. Side View of Fluorometric Comparator

1. Sliding test tube holder
2. No. 5874 H.R. red purple ultra filter
3. Mercury vapor lamp, Type B-H-4

standardized gravimetrically. This solution was diluted 1 to 1000 to give the working standard.

A standard beryllium solution was also prepared from a high-grade beryl of known beryllium content by the following procedure: A 500-mg. sample was fused with 10 grams of fusion mixture, the melt dissolved in 150 ml. of 1 to 1 hydrochloric acid, and diluted to 250 ml. This solution was further diluted 1 to 20 to give a working standard, 1 ml. of which contained 11.2 micrograms of beryllium oxide. The weaker solution was used almost exclusively as a standard in the analysis of unknowns. A new standard solution was prepared about once a month, as silica separated from the concentrated solution after standing some time. The diluted solutions were unaffected by standing.

Equipment. In addition to the ordinary glassware, pipets, burets, and platinum ware common to all laboratories, the following pieces of equipment were used:

Two 2-ml. automatic pipets.

Pyrex test tubes (20 × 200 mm.), calibrated at 12.5 and 25 ml. These are sold as Lewis-Benedict blood sugar tubes. When used for visual comparison, only those tubes were selected which were the same height (within ±3-mm. tolerance) from the bottom up to the 25-ml. graduation.

Optical glass cell (for photoelectric determination only), 31-mm. cube inside measurement, 1-mm. wall thickness. These may be purchased from Pyrocell Manufacturing Co., 207 East 84th St., New York 28, N. Y.

Glass standards (for photoelectric determination only). A series of glass standards was made by embedding 2.5 × 2.5 cm. squares of fluorescent glass in a horizontal position in wooden blocks which were the same size as the optical cell. The glass squares were prepared by cutting a 5-cm. (2-inch) square Corning filter No. 3750 into quarters. The faces next to the phototube were covered by a section of Corning filter No. 3480, to transmit light of the desired wave length. The sides of the block next to the phototube and the light source, respectively, were covered with brass plates. The apertures in these plates were made of such size that readings were obtained comparable to those resulting from the beryllium standards.

Visual comparator. A visual comparator similar to that used by Palmes and Alford (*3*) was built from wood and painted a flat black with a nonfluorescent paint. Figure 1 shows the light-tight comparison box, which was equipped with a sliding rack to carry four sample tubes. Ultraviolet light was admitted directly behind the test tube rack through a Corning heat-resistant red purple ultra filter No. 5874, which eliminated visible light. The fluorescence was observed through the viewing aperture in the front of the box. A hinged panel immediately above the tube rack facilitated rapid changing of unknowns and standards. The light source shown in Figure 2 was a General Electric Type B-H-4 high-pressure mercury lamp with an aluminum reflector. An auxiliary ballast transformer with a primary of 115 volts was needed for the mercury lamp. This complete light source unit may be purchased from the General Electric Vapor Lamp Co., Hoboken, N. J. All the samples analyzed during this work were matched in this comparator although many were also run photoelectrically.

Photoelectric fluorometers. The original fluorometer used in the early part of the work was built in the laboratory of one of the authors (C. E. White) according to the description of Hand (*2*), except that a galvanometer with a sensitivity of 1 scale division equivalent to 0.0004 microampere was substituted for the microammeter and a voltage regulator and a ventilating fan were used to control the lamp voltage and temperature.

All later data, including those in Table II, were taken with a Beckman spectrophotometer for which a fluorometric attachment was designed (*1*).

A Lumetron fluorescence meter could also be used.

ANALYTICAL METHOD

If the mineralogical composition and approximate beryllium content of the sample are not known, a preliminary test is made to indicate which of the procedures described below is to be followed in preparing the beryllium-bearing solution.

For the test run the sample is treated as though it contained over 1% beryllium oxide. Aliquots of 0.1 and 1.0 ml. are then used to develop the fluorescence as described under the section for the determination of beryllium oxide in solutions. Only 5 standards (0.0, 0.5, 1.0, 1.5, and 2.0 ml. of standard solution) are used for this comparison. If a photoelectric instrument is employed, no standard solutions are required; the instrument is set with a glass standard and the readings are compared with a previously prepared standard curve.

Preparation of Samples for Analysis. Procedure For Ores of Beryllium Oxide Content Greater Than 1% (low in interfering ions.) Mix a 500-mg. sample with 10 grams of fusion mixture in a 100-ml. platinum dish. Fuse at 1000° C. in a muffle furnace for 3 to 5 minutes. Remove the dish from the furnace, cool somewhat, and place in a 600-ml. beaker which contains 150 ml. of 1 to 1 hydrochloric acid. When solution is complete, which usually requires about 5 minutes, remove and rinse the platinum dish. Cool the solution to room temperature, transfer to a 250-ml. volumetric flask, and add water to 250 ml. Make a test run on this solution to indicate necessary dilution, and dilute so that 1 ml. of solution is equivalent to approximately 10 micrograms of beryllium oxide. Use four aliquots which cover a range of 5 to 24 micrograms of beryllium oxide for the analysis.

Procedure for Ores of Beryllium Oxide Content Less Than 1% (low in interfering ions). Moisten a 1.000-gram sample contained in a 100-ml. platinum dish with aerosol and distilled water. Add 30 to 40 ml. of hydrofluoric acid, 3 ml. of nitric acid, and 1 ml. of sulfuric acid. Evaporate to dryness on a hot plate, and ignite over a burner until the sulfur trioxide fumes are expelled. Add 10 grams of the fusion mixture and fuse in a muffle furnace. Remove from the furnace, cool several minutes, place the dish and contents in a 600-ml. beaker, add 100 ml. of 30% hydrochloric acid, and heat on a steam bath until the melt is dissolved. Columbium and tantalum, if present, will hydrolyze and separate at this point. When this occurs remove and rinse the platinum dish, boil the solution 0.5 hour, filter, and wash the precipitate well with water. In the absence of columbium and tantalum filtration is unnecessary.

If the beryllium oxide content is 0.1% or more, transfer the solution into a 250-ml. volumetric flask and make up to volume. Make a test run to determine whether dilution is necessary, and if so, dilute until 1 ml. contains approximately 10 micrograms of beryllium oxide. Use four aliquots of original or diluted solution, which contain 5 to 24 micrograms of beryllium oxide for the analysis.

When the beryllium oxide content is less than 0.1%, concentration is necessary. To do this, heat the solution until it boils and precipitate beryllium and aluminum with ammonium hydroxide at a pH of 8.0. Boil 1 minute and set aside for at least 2 hours or preferably overnight. Filter on Whatman No. 41 H paper. Wash several times with 0.1% ammonium hydroxide and once with water. Return paper and precipitate to original beaker, dissolve the precipitate in 5 ml. of concentrated hydrochloric acid and about 25 ml. of water, filter into a 100-ml. volumetric flask, wash paper well with water, and dilute the solution to 100 ml.

Make a test run to determine whether further dilution is necessary, and if so, dilute until 1 ml. contains approximately 10 micro-

rams of beryllium oxide. Use aliquots of original or diluted solution, which contain 5 to 24 micrograms of beryllium oxide for the analysis.

PROCEDURE FOR ORES WITH HIGH IRON, MANGANESE, AND MAGNESIUM CONTENT. Treat as directed in the preceding section to the step where the sample is in solution and columbium and tantalum are removed. At this point add 0.5 ml. of concentrated nitric acid and heat to boiling. Add 20% sodium hydroxide until precipitation starts, and then add at one time a volume of 20% sodium hydroxide equivalent to one half of the volume of the neutralized solution. Add about 1 gram of sodium peroxide. Boil 0.5 hour, cool 15 minutes, and filter with suction on a heavy pad of filter-paper pulp prepared from Whatman No. 41 H paper. Wash well with hot 5% sodium hydroxide and twice with water. Acidify the filtrate with hydrochloric acid, bring to a boil, and precipitate beryllium and aluminum with ammonium hydroxide at a pH of 8.0. Boil 1 minute and set aside for at least 2 hours or preferably overnight. Filter with suction on a Whatman No. 41 H filter paper and continue as directed in the preceding method.

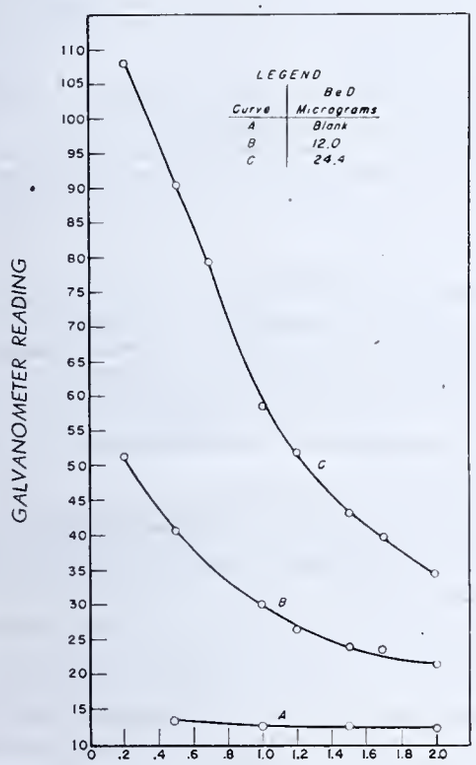

Figure 3. Effect of Dye Concentration on Fluorescence of Beryllium and Quinizarin

PROCEDURE FOR DETERMINATION OF BERYLLIUM OXIDE IN SOLUTIONS. Preparation of tubes for the development of fluorescence should follow rigidly the standard procedure because small variations in the preparative routine frequently lead to off-color solutions.

Prepare a set of standards in twenty-three calibrated tubes which contain the following volumes of standard beryl solution, or beryllium chloride solution, 1 ml. of which contains an equivalent of 10 to 12 micrograms of beryllium oxide: five blank tubes, 0.2, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, and 2.0 ml. Pipet four aliquots of each unknown sample which contain 5 to 24 micrograms of beryllium oxide into similar tubes. Add 2 ml. of 5% sodium citrate from an automatic pipet and 3 drops of quinizarin to each tube. Neutralize with 2% sodium hydroxide until the color of the solution changes to purple. Add water to 12.5-ml. mark. Add 1 ml. of 0.029% quinizarin to each tube and mix by swirling. If any of the solutions are incompletely neutralized, as indicated by a light color, add more sodium hydroxide until they match the color of the standards. Add 2 ml. of 1% sodium hydroxide from an automatic pipet, and water to 25 ml. Starting with the blank tubes, cover the end of the tubes with the thumb, and shake well to mix. Glass-stoppered containers may be used, but rubber stoppers must not be used in these tubes under any circumstances, because the action of acetone on the rubber produces a brilliant blue fluorescence. The solutions even dissolve a less brilliant blue fluorescent substance from the skin; therefore, the same thumb should be used to shake all the tubes and it should be well rinsed before starting. The blank solutions remove most of the material from the thumb, so that the standards and samples are unaffected. Should it prove necessary to make up a single tube, the mixing may be effected by pouring the solution back and forth from two test tubes, or by preceding it with four blank solutions.

All solutions should be protected from sunlight or the direct light from an electric lamp; however, it is not necessary to work in darkness.

Compare the unknown solutions with the standard solutions in the visual comparator, or make readings with a photoelectric fluorometer and calculate the percentage of beryllium oxide.

EXPERIMENTAL RESULTS AND DISCUSSION

The method was tested on four beryllium ores. These samples were analyzed for beryllium oxide by the visual fluorescent method; also, representative samples were sent to seven different laboratories experienced in beryllium analysis. The average results obtained by these seven laboratories were considered as representing most nearly the true beryllium content of the ores. The results are given in Table I, which also shows the reproducibility of the fluorescent method.

Table II shows the results of analyses by the fluorometric method on standard mixtures of known beryllium oxide content. These were prepared from the high-grade sample by dilution with beryllium-free potash feldspar. The errors on the mixture that contained 0.022% beryllium oxide, although somewhat large in a relative sense, are within acceptable limits because in practice the third figure would not be shown; with one exception, all

Table I. Determination of BeO in Analyzed Samples

Sample	BeO Found: Mean and standard deviation of mean of results from seven laboratories[a]	BeO Found: Visual fluorometric method
	%	%
Beryl concentrate	11.2 ± 0.1	11.1 11.2 11.1 11.2
Medium-grade beryl ore	5.51 ± 0.07	5.36 5.51 5.38 5.36
Low-grade beryl ore	1.39 ± 0.05	1.38 1.39 1.36 1 39
Tailing waste	0.26 ± 0.02	0.26 0.25 0.24 0.26

[a] Geological Survey, Bureau of Mines, Rolla, Mo., Salt Lake City, Utah, and College Park, Md., Paul Brinton, Pasadena, Calif., Beryllium Corp. of Pennsylvania, Temple, Pa., and Ledoux and Co., New York, N. Y.

Table II. Determination of BeO in Standard Mixtures by Fluorometric[a] Method

(Comparison of visual and photoelectric methods)

BeO Present	BeO Found: Visual method	BeO Found: Photoelectric method
%	%	%
2.24	2.28 2.27 2.29 2.31	2.31 2.27 2.29 2.26
1.12	1.14 1.12 1.13 1.12	1.12 1.15 1.09 1.16
0.22	0.23 0.22 0.24 0.22	0.24 0.23 0.23 0.23
0.022	0.019 0.020 0.019 0.016	0.024 0.026 0.023 0.020

[a] For each concentration of beryllium oxide, four different samples were carried through entire procedure.

would be reported as 0.02%. It is virtually impossible to analyze samples which contain such low amounts of beryllium oxide by gravimetric procedures. Table II also gives a comparison of results by the visual and photoelectric methods and shows them to be in close agreement. The choice of procedure need be governed only by personal preference or the availability of a photoelectric instrument.

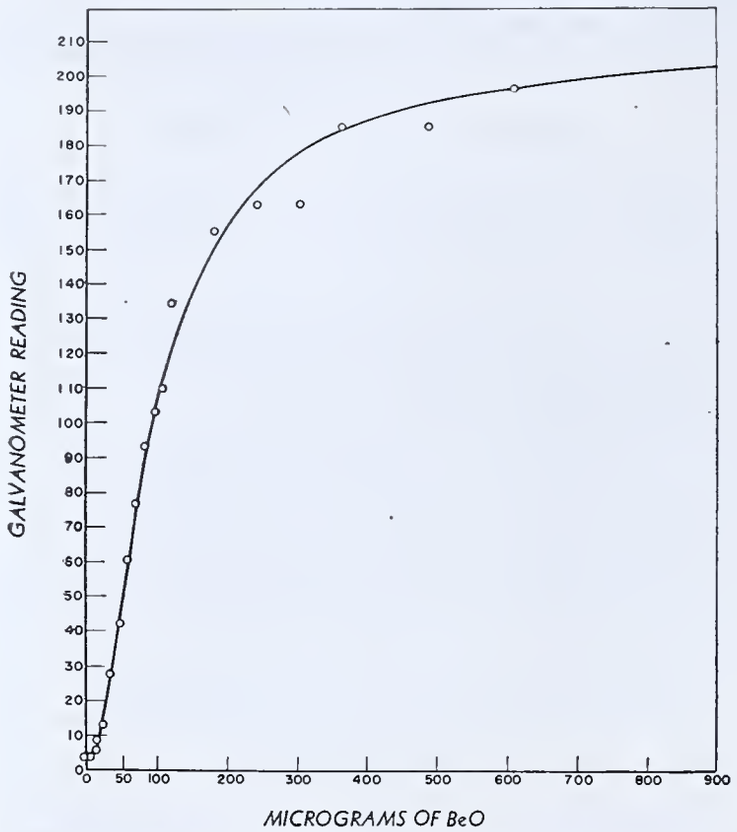

Figure 4. Effect of Beryllium Concentration on Fluorescence of Beryllium and Quinizarin

Characteristics of the Fluorescence. Beryllium reacts with quinizarin over a wide range of concentration and produces a fluorescent band which covers the spectral range from 5700 to 6400 Å. One milliliter of 0.029% quinizarin with 5 to 24 micrograms of beryllium oxide appeared to be the best ratio for visual comparison. Figure 3 shows the effect of changes in the dye concentration on the fluorescence and indicates the necessity for careful control of the amount of dye used.

Figure 4 shows the relation between the beryllium concentration and the fluorescence of the solution. Even though the use of the straight-line portion of the curve between 25 and 90 micrograms of beryllium oxide would seem desirable, this is not generally practical except for pure beryllium solutions when measured photoelectrically.

Effect of Other Ions. All elements that are precipitated in an alkaline citrate solution must be removed before beryllium is determined by the fluorometric method. Among these are iron, manganese, magnesium, calcium, and silica, all of which, when present in sufficient quantities, decreased the intensity of the fluorescence. Strong oxidizing ions such as hexavalent chromium interfered through destruction of the dye. For the most part, the presence of these substances in amounts sufficient to interfere are indicated by off-color fluorescence.

The weights of the ions given in the following section refer to the amount of the various substances contained in one comparison tube.

Silica, when present to the extent of 1.2 mg., caused the fluorescence to pale, and the silica separated as a gelatinous precipitate which adhered to the walls of the tube. When 4.0 mg. or more were present the precipitate settled to the bottom of the tube. It appears that more silica can be tolerated as sodium silicate than as a complex ion with boron.

Iron, in amounts greater than 0.04 mg. of ferric oxide, produced a dull and darker red fluorescence than pure beryllium solutions. When more than 0.6 mg. of ferric oxide was present ferric hydroxide settled out. During the neutralization step, solutions which contained appreciable amounts of iron assumed a maroon and then a brownish color.

Manganese, when present in quantities greater than 0.02 mg., caused a decrease in the intensity of fluorescence. A dark-red shade characterized the color of the fluorescence when 0.2 mg. or more of manganese was present. Solutions which contained more than this had a murky appearance due to a fine suspended precipitate.

Magnesium, when present in amounts exceeding 0.06 mg. of magnesium oxide, produced a fluorescence that was sometimes brighter when first prepared but soon faded to pale lavender. Formation of a purple flocculent precipitate started at 0.1 mg. of magnesium oxide and absorbed all the dye from the solution. Excessive amounts of magnesium oxide (30 to 40 mg.), which produced a heavy precipitate of magnesium hydroxide, gave a brilliant fluorescence that could be seen in ordinary light. The presence of magnesium can be recognized during the neutralization step because the solutions have a more blue-purple color than the regular samples.

Calcium, when present in excess of 1.0 mg. of calcium oxide, caused a paler and more lavender fluorescence. The solutions themselves had a clear slate-blue color.

Lithium produced a fluorescence with quinizarin that was somewhat more scarlet than that obtained with beryllium. If present in quantities greater than 0.04 mg. of lithium oxide in beryllium-bearing solutions, it enhanced the fluorescence of beryllium.

Aluminum, when present to the extent of 10 to 60 mg. of aluminum oxide, caused an increase in the intensity of fluorescence but a decrease in redness. A pink-salmon color appeared before neutralization was complete. It is recommended in such cases that a solution which contains less aluminum be used as a control and the neutralization continued until the color of the two solutions is the same. The precipitate formed in the high aluminum solutions may be recognized by its white bulky nongelatinous appearance.

Zinc, in amounts greater than 0.5 mg., caused a decrease in the fluorescence of beryllium and quinizarin; amounts greater than 1.0 mg. of zinc were precipitated. The zinc precipitate appeared first as a fine white suspension on neutralization, which flocculated into large white flocs.

Phosphate, when present in amounts less than 30 mg. of phosphorus pentoxide, produced no noticeable effect.

Hydroxyl. Excess sodium hydroxide caused a less intense fluorescence and a darker purple solution. Care should be taken during the neutralization step not to exceed the end point.

Fluoride, in amounts remaining after the hydrofluoric acid treatment, caused no deleterious effect.

Sulfate. Sulfuric acid of the same normality as the hydrochloric acid may be substituted for the latter in the procedures.

The precautions that must be observed in almost all fluorometric analyses are applicable. For example, technical-grade acetone has a decided blue fluorescence and cannot be substituted for the C.P. reagent. All commercial alcohol on hand in this laboratory also had a blue fluorescence, although if redistilled from an all-glass apparatus it could be substituted for acetone. The dye solution and solutions prepared for comparison must be kept from all contact with rubber and grease. On short

Table III. Effect of Sodium Hydroxide Separation of 20 Mg. of Ferric Oxide on Recovery of Beryllium Oxide

Solution No.	BeO Originally Taken	BeO in Diluted Solution		Error
		Present	Found	
	Mg.	*Micrograms/ml.*		%
1	0	0	0	...
2	0.122	1.22	0.77	−36.1
3	0.610	6.10	5.52	− 9.4
4	1.22	12.2	10.6	−13.1
5	1.83	9.15	8.74	− 4.5
6	2.44	12.2	12.4	+ 1.6
7	4.88	9.76	9.74	− 0.21
8	7.32	7.32	7.73	+ 5.6
9	9.76	9.76	10.5	+ 7.2
10	12.2	4.88	5.10	+ 4.5
11	24.4	9.76	9.91	+ 1.5
12	36.6	7.32	6.65	− 9.1
13	61.0	12.2	12.1	− 0.82
14	85.4	8.54	8.34	− 2.3

contact, acetone extracts a substance from rubber that produces a brilliant blue fluorescence. Grease, as on stopcocks, is also fluorescent and should never be used with acetone solutions and only sparingly with the other solutions. Rubber tube connections may be used for the sodium citrate and sodium hydroxide solutions.

Separations. The composition of the beryllium ores is such that iron, manganese, and silica are frequently present in low-grade materials in amounts exceeding the tolerable limits. Silica in the gangue materials is removed by the preliminary hydrofluoric acid treatment used for all low-grade materials. The iron, manganese, and all other ions mentioned except aluminum are removed by the sodium hydroxide and ammonium hydroxide treatments. These separations were adopted after laboratory tests indicated that they could be safely employed.

Experiments were made to test the effects of the sodium hydroxide separation on the beryllium recovery (Table III). Fourteen solutions were prepared which contained the equivalent of 0 to 85 mg. of beryllium oxide, 8 mg. of aluminum oxide, 20 mg. of ferric oxide, and 10 grams of fusion mixture. These solutions were treated by the method described for samples with a high iron and manganese content, and the beryllium present was determined after treatment for the separation of iron and proper dilution. The readings were made in the visual comparator.

The results of these experiments were considered well within the experimental limits. The percentage error was always less than 10%, except when the amount of beryllium oxide present was less than 1.22 mg. This amount of beryllium oxide would represent 0.12% BeO if it occurred in a 1-gram sample; hence it is evident that the seemingly large errors which appear for the first three solutions in Table III can easily be tolerated, since in the analysis of samples they would make very little difference in the final answer.

To test the sodium hydroxide separation for larger amounts of iron, a series of nine solutions was prepared. Each solution contained the equivalent of 4.88 mg. of beryllium oxide, 8 mg. of aluminum oxide, 10 grams of fusion mixture, and 75 ml. of 30% hydrochloric acid. Iron in the form of the chloride was added in the amounts shown in Table IV.

Iron was separated as above and the beryllium content determined. The amounts of beryllium oxide found by visual comparison were a little lower than the amount present, but all were within 7.5% of the true value. The results are summarized in Table IV.

Table IV. Effect of Separation of Large Amounts of Iron on Recovery of Beryllium Oxide

Solution No.	Fe_2O_3 Added	BeO in Diluted Solution		Error
		Present	Found	
	Mg.	*Micrograms/ml.*		%
1	20	9.76	9.49	−2.8
2	30	9.76	9.31	−4.6
3	40	9.76	9.80	−0.41
4	50	9.76	9.24	−5.3
5	60	9.76	9.37	−4.0
6	80	9.76	9.31	−3.6
7	100	9.76	9.04	−7.4
8	150	9.76	9.05	−7.3
9	200	9.76	9.21	−5.6

It can be concluded from these experiments that sodium hydroxide can be used satisfactorily to separate iron from beryllium wherever it may occur in amounts sufficient to interfere with the analysis.

Should the aluminum concentration ever exceed the tolerable limits, the bulk of it may be separated as the chloride in ether by the Gooch Havens method. This procedure was tried on the synthetic sample which contained 0.22% beryllium oxide and to which aluminum was added. Excellent results were obtained and there was no loss of beryllium.

Effect of Light. Tubes prepared for comparison of the fluorescence must be protected from sunlight and the direct light from an electric lamp. Upon exposure to strong light, the purple color faded, first with an increase in the intensity of the fluorescence followed by complete destruction of the color and fluorescence.

Effect of Temperature. Small temperature changes as experienced under normal room conditions appear to have no appreciable effect on the fluorescence of beryllium and quinizarin, and any changes which might occur are offset by preparation and comparison of standards and unknowns at the same time. Large temperature changes of 15° to 20° do affect the fluorescence. A decrease in temperature is accompanied by increase in the intensity of the fluorescence.

Effect of Time. The solutions prepared for fluorometric comparison may stand approximately 4 hours before the fluorescence is measured without introduction of an appreciable error; after longer periods of time, the purple color fades slowly and the fluorescence is increased.

Table V. Determination of Beryllium Oxide in Minerals Other Than Beryl

(Comparison of gravimetric and fluorometric methods)

Mineral	% BeO Found	
	Gravimetric	Fluorometric
Chrysoberyl	16.5	17.1
Helvite	5.36	5.35
Phenacite	44.6	44.2

APPLICATION TO OTHER MINERALS

Minerals other than beryl may be analyzed for beryllium by the procedures given in this paper. Samples of phenacite, helvite, and chrysoberyl were analyzed by the gravimetric and fluorimetric methods (Table V). The agreement between the two methods was of the same order as that obtained with the standard samples. Phenacite and helvite behaved like beryl and caused no difficulty. Chrysoberyl, however, required a prolonged fusion (30 minutes) followed by solution, filtration, and a second fusion of the residue. Two fusions gave complete solution.

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Determination of Inorganic Phosphorus in Plant Materials

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A method is described for the determination of inorganic phosphorus in a variety of plant materials, including those high in protein. A trichloroacetic acid extraction and colorimetric evaluation by a modification of the Berenblum and Chain procedure are utilized. The molybdenum blue complex developed in isobutyl alcohol has absorption maxima at 625–630 and 730 mμ, respectively, and is stable for 19 hours.

A METHOD for determining inorganic phosphorus in plant materials was required in investigations on the influence of the phosphorus compounds present on the preparation of oilseed proteins for industrial utilization. It was essential that the method be unaffected by large amounts of protein or by colored and turbid extracts. After consideration of available methods, a trichloroacetic acid extraction of inorganic phosphorus and colorimetric analysis by a modified Berenblum and Chain (*2*) procedure were selected for study. Certain details of the colorimetric method have been improved; the procedure has been adapted to use with either a spectrophotometer or photoelectric colorimeter; and the validity of the method has been investigated.

REAGENTS

Trichloroacetic acid solution, 0.75 *N*. Dissolve 123 grams of reagent grade acid in water, and make up to 1 liter. Make the reagent as needed, or store in a refrigerator.

Molybdate reagent. Dissolve 50 grams of ammonium molybdate, $(NH_4)_6Mo_7O_{24}.4H_2O$, in 400 ml. of 10 *N* sulfuric acid and 500 ml. of water, make up to 1 liter, and store in a paraffin-lined bottle.

Sulfuric acid, approximately *N*. Dilute 114 ml. of concentrated sulfuric acid to 4 liters.

Stannous chloride stock solution, 10 grams of stannous chloride hexahydrate dissolved in 25 ml. of concentrated hydrochloric acid. Store in a small glass-stoppered brown bottle.

Stannous chloride, dilute solution. Dilute 1 ml. of stock solution to 200 ml. with approximately *N* sulfuric acid just before use.

Isobutyl alcohol. Commercial grade, with a boiling range 106° to 110° C., is satisfactory.

Ethyl alcohol, 95%.

Standard phosphate solution. Recrystallize A.C.S. grade monobasic potassium phosphate three times from water, dry at 110° C., and store in a desiccator over concentrated sulfuric acid. Dissolve 4.3929 grams of dry salt in 300 ml. of water and 200 ml. of approximately *N* sulfuric acid. Add a few drops of 0.1 *N* potassium permanganate as preservative and make up to 1 liter with water. This stock solution, 1.0 mg. of phosphorus per ml., is stable. Dilutions of the stock solution are made as needed.

ANALYTICAL PROCEDURE

Weigh 1-gram samples of the ground material, 60-mesh or finer into 125-ml. glass-stoppered Erlenmeyer flasks; add exactly 50 ml. of 0.75 *N* trichloroacetic acid; and shake mechanically for 1 hour. Filter through ashless filter paper of medium retentivity, discarding the first portion of the filtrate.

Pipet aliquots containing from 0.005 to 0.045 mg. of inorganic phosphorus (usually 2 ml.) into 125-ml. separatory funnels with a mark at 20 ml. Add 5 ml. of the molybdate–sulfuric acid reagent and distilled water to the 20-ml. mark. Add 10 ml. of isobutyl alcohol and shake for 2 minutes. Discard the aqueous layer, and wash by shaking once with 10 ml. of approximately *N* sulfuric acid. Add 15 ml. of dilute stannous chloride, shake for 1 minute, then discard aqueous layer. Transfer the blue isobutyl alcohol layer to a 50-ml. volumetric flask, washing the funnel with ethyl alcohol. Make to volume with ethyl alcohol. Determine the transmission of the blue solutions against a blank containing all reagents with an Evelyn photoelectric colorimeter, using the No. 720 filter or a spectrophotometer at 730 mμ at any time from 40 minutes to 19 hours after color development.

Prepare a calibration curve by pipetting known concentrations of inorganic phosphorus in the range 0 to 0.045 mg. of phosphorus, obtained by diluting stock phosphate solution, into 125-ml. separatory funnels and developing the color exactly as outlined in the procedure. The logarithms of the per cent transmission values of the standards may be fitted to a straight line by the method of least squares and the calculations for unknowns made from the equation of this line (*7*). The calibration curve once determined for any one instrument need not be repeated, since the standard curve has been found to be reproducible.

An alternate calibration method for the Evelyn colorimeter consists of determining the K_2 constant from the logarithms of the transmission values of the standards. Values for K_2 determined with the Evelyn colorimeter (No. 720 filter) are shown in Table I. When using the Beckman spectrophotometer calculations may be made from the extinction coefficient. The extinction coefficient α at 730 mμ, $\alpha = E^{g/L}_{1\text{ cm.}}$, was found to be 523 using the Beckman spectrophotometer. The concentration of phosphorus, in mg., in the final 50-ml. volume is obtained by the equation:

$$\text{Mg. of P in 50-ml. volume} = \frac{\text{optical density}}{\alpha L} \times 50$$

where L = cell length in cm.

Table I. Calibration Data for Evelyn Colorimeter

Mg. of P in 50-Ml. Volume	Evelyn Colorimeter, 720 Filter: % transmission	Log % transmission	K_2[a]
0.005	80.2	1.9042	0.0521
0.010	64.7	1.8109	0.0529
0.015	52.2	1.7177	0.0531
0.020	41.7	1.6201	0.0526
0.025	33.8	1.5289	0.0531
0.030	27.5	1.4393	0.0535
0.035	22.5	1.3522	0.0540
0.040	18.3	1.2625	0.0542
0.045	15.0	1.1761	0.0546
			Av. 0.0533

Mg. of P = (2-log transmission) × K_2

[a] $K = \frac{\text{2-log transmission}}{\text{mg. of P in 50-ml. volume}}$; $K_2 = \frac{1}{K}$

INVESTIGATION OF EXTRACTION PROCEDURE

Most inorganic phosphorus methods for plant material utilize a dilute sulfuric or hydrochloric acid extraction of inorganic phosphorus (*3*, *6*, *11*). Alcohol acidified with hydrochloric acid has also been used (*5*). For material high in protein, however, extraction with dilute sulfuric or hydrochloric acid is unsatisfactory, since enough protein is extracted to cause a precipitate to form upon addition of ammonium molybdate in the colorimetric analysis, unless this protein is precipitated before analysis. Trichloroacetic acid was found satisfactory in the present method since, in the concentration used, it does not extract protein precipitable by the molybdate reagent and may be assumed to extract only nonprotein nitrogen.

In an experiment on this phase of the method, 1-gram samples of Skellysolve F–extracted peanut meal were extracted for 1 hour with trichloroacetic acid in which the concentration varied from 0.1 to 2.0 *N*. Within the concentration range 0.37 to 1.0 *N*, the amount of inorganic phosphorus extracted, 0.71 mg., was constant. One-gram samples of the same meal were also extracted for 1 hour with both 0.36 *N* and *N* sulfuric acid and 0.55 *N* and *N* hydrochloric acid, protein being precipitated from the filtrates by making them 0.75 *N* with respect to trichloroacetic acid before analysis. The same amount of inorganic phosphorus, 0.71 mg., extracted in each case, was equal to that extracted by trichloroacetic acid in the concentration range 0.37 to 1.0 *N*. Experience with a variety of plant materials has shown 0.75 *N* trichloroacetic acid to be satisfactory for the extraction.

Extraction time was investigated by extracting 1-gram samples of a different solvent-extracted peanut meal for periods ranging from 0.5 to 4 hours with 0.75 *N* trichloroacetic acid. The amount of inorganic phosphorus extracted, 0.69 mg., was practically constant over the entire time range, the only effect of increasing the time being the extraction of a little more organic phosphorus as determined by difference between the inorganic and total phosphorus contents of the extracts. The constancy of the inorganic phosphorus values with increasing extraction time is evidence that there was no conversion of organic to inorganic phosphorus during the 1-hour extraction of the plant materials investigated.

RECOVERY OF ADDED INORGANIC PHOSPHORUS AND STUDY OF INTERFERENCES

Satisfactory recovery of inorganic phosphorus added to peanut and cottonseed meals in the form of monobasic potassium phosphate, KH_2PO_4, and primary calcium phosphate, $Ca(H_2PO_4)_2.H_2O$, was obtained as shown in Table II. Satisfactory recovery of inorganic phosphorus added to highly colored trichloroacetic acid extracts of goldenrod, sweet potato, and tobacco leaves was also obtained. Tricalcium phosphate, $Ca_3(PO_4)_2$, is also soluble in 0.75 *N* trichloroacetic acid.

No interference due to colored extracts has been observed. Chlorophyll is not extracted by 0.75 *N* trichloroacetic acid. The yellow color of most plant extracts did not pass into the isobutyl alcohol and consequently was discarded with the aqueous phase. In cases where color was extracted by the isobutyl alcohol, examples being cottonseed meal, wheat straw, goldenrod, and sweet potato leaves, the extracted color had no absorption at 730 mμ and consequently did not interfere. Blanks run on all the above colored extracts, adding 5 ml. of 4 *N* sulfuric acid instead of the acid molybdate reagent, gave transmission values ranging from 99.5 to 100% when read against 10 ml. of isobutyl alcohol made up to 50 ml. with 95% ethyl alcohol.

With a few plant materials, notably cottonseed, turbid solutions were obtained on extraction with 0.75 *N* trichloroacetic acid. Shaking with isobutyl alcohol and washing once with *N* sulfuric acid in the method cleared these extracts. No turbidity has been encountered in the final blue solutions.

Table II. Recovery of Inorganic Phosphorus Added to Petroleum Ether–Extracted Peanut and Cottonseed Materials

Material, 0.5 Gram	Inorganic Phosphorus In sample	Added	Total	Found	Recovered
	Mg.	*Mg.*	*Mg.*	*Mg.*	%
Peanut kernels	0.378	0.500[a]	0.878	0.883	101
Peanut testa	0.180	0.500[a]	0.680	0.673	99
Cottonseed meal	0.301	0.500[a]	0.801	0.795	99
Peanut kernels	0.365	2.255[b]	2.620	2.600	99
Cottonseed meal	0.390	2.458[b]	2.848	2.800	98

[a] Added as KH_2PO_4.
[b] Added as $Ca(H_2PO_4)_2.H_2O$.

Most of the trichloroacetic acid extracts of the plant materials investigated contained reducing agents which caused a partial reduction to molybdenum blue prior to the addition of stannous chloride, the extent varying considerably with different plant materials. Although this premature reduction does not affect the precision of the method, inasmuch as a large excess of stannous chloride is used to develop the maximum molybdenum blue color, it might affect those inorganic phosphorus methods in which the concentration of reducing agent must be carefully controlled.

To test the applicability of the method in the presence of organic phosphorus compounds, 1-gram samples of solvent-extracted peanut meal were weighed, and 25 mg. of the various organic phosphorus compounds listed in Table III were added, and carried through the analytical procedure. Suitable controls for the meal alone and for each phosphorus compound were also run. The samples were allowed to stand at room temperature for 24 hours, and were then again analyzed for inorganic phosphorus.

Table III. Stability of Organic Phosphorus Compounds Added to 1-Gram Samples of Solvent-Extracted Peanut Meal under Method Conditions

Organic Phosphorus Compound Added, 25 Mg.	Inorganic Phosphorus Present In meal	In added compound	Total	Inorganic Phosphorus Found 1-hour extraction	24-hour extraction at 25° C.
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*
Na-β-glycerophosphate	...	0.000	0.000	0.000	0.000
Na - β - glycerophosphate + meal	0.723	0.000	0.723	0.723	0.745
Di - K - glucose - 1-phosphate	...	0.019[a]	0.019	0.228	1.388
Di - K - glucose - 1-phosphate + meal	0.723	0.019	0.742	0.915	1.983
Fructose diphosphate	....	0.015[a]	0.015	0.025	0.145
Fructose diphosphate + meal	0.723	0.015	0.738	0.750	0.883
Adenylic acid	...	0.000	0.000	0.000	0.000
Adenylic acid + meal	0.723	0.000	0.723	0.720	0.745
Peanut phytin	...	0.032	0.032	0.028	0.028
Peanut phytin + meal	0.710	0.032	0.742	0.745	0.770

[a] Calculated from inorganic phosphorus analysis by shakeout method and extrapolation to zero time.

From the results shown in Table III it is evident that glucose-1-phosphate is rapidly hydrolyzed by the 0.75 *N* trichloroacetic acid used in the extraction step. Fructose diphosphate is hydrolyzed very slowly, while sodium β-glycerophosphate, adenylic acid, and phytin show no hydrolysis after 24 hours in contact with the acid. Glucose-1-phosphate is said to be involved in one step of plant carbohydrate metabolism through phosphorylation; and the determination of inorganic phosphorus is frequently used to follow the progress and direction of phosphorylation, with glucose-1-phosphate as a substrate (*4*, *10*).

To determine the hydrolysis of glucose-1-phosphate in the shakeout procedure, 10-mg. samples of the dipotassium salt of glucose-1-phosphate were added to a series of separatory funnels along with 5 ml. of the acid-molybdate reagent. The samples were analyzed after standing for various periods determined from the time the molybdate was added to the time at which the shaking with stannous chloride was completed. The shortest interval, 4 minutes, showed 0.07% hydrolysis of glucose-1-phosphate, 5 minutes 0.13%, 10 minutes 0.37%, 28 minutes 0.97%, and 67 minutes 2.87% hydrolysis. The 5-minute value of 0.13% hydrolysis compares favorably with that found by Hanes (*10*), who, with the method of Allen (*1*), obtained 0.4% hydrolysis of glucose-1-phosphate in 5 minutes' contact with 0.77 *N* perchloric acid.

No increase in inorganic phosphorus was found after the developed colored solutions were allowed to stand overnight, showing that complete separation of inorganic phosphorus from glucose-1-phosphate was obtained in the shakeout method. The shakeout method should prove valuable when it is used to follow enzymatic synthesis by measuring increase or decrease in inorganic phosphorus. An almost insignificant amount of hydrolysis of glucose-1-phosphate takes place in the approximately 5 minutes needed for analysis; and, once developed, the colorimetric readings may be taken at any time up to 19 hours after development. The time factor, which must be carefully controlled in the usual colorimetric methods when glucose-1-phosphate is present, is thereby eliminated.

Inasmuch as compounds such as glucose-1-phosphate and fructose diphosphate were found to be hydrolyzed by 0.75 *N* trichloroacetic acid in the extraction step, 1-gram samples of the plant materials listed in Table IV were simultaneously extracted for 24 hours at room temperature and 24 hours at 5° C., after which the extracts were analyzed for inorganic phosphorus.

Although from the results in Table IV it is evident that easily hydrolyzable organic phosphorus compounds do not appear to be present in any significant amount in the plant materials investigated, with other materials 1-hour extraction should be compared with overnight extraction to establish the absence of these compounds.

EVALUATION OF MOLYBDENUM BLUE COMPLEX

Spectrophotometric curves of the molybdenum blue complex developed by the shakeout method for 0.030 mg. of inorganic phosphorus and for extracts of peanut and cottonseed meals of approximately the same phosphorus concentration are shown in Figure 1. A Beckman quartz spectrophotometer with a 1-cm. cell was used, a reagent blank being the reference solution. All three curves show the same two points of maximum absorption at 625–630 mμ and at 730 mμ, which are characteristic. The maximum at 730 mμ is much sharper than that at 625–630 mμ, and measurements should be made at 730 mμ to obtain maximum sensitivity. That the position of the maxima is unchanged by the presence of plant extracts, as shown in Figure 1, is evidence that materials extracted from plants do not interfere with the determination by the shakeout method. In another experiment with the same concentration of phosphorus, dilution to volume with isobutyl alcohol did not change the position of the two maxima, proving that these are not due to the influence of ethyl alcohol. The use of isobutyl alcohol, however, intensifies the color and decreases transmission at 730 mμ by about 7%, which demonstrates that the ratio of isobutyl to ethyl alcohol should be kept constant in the method.

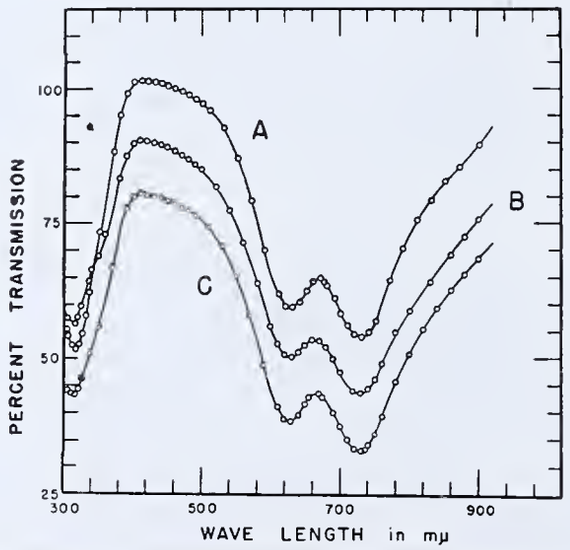

Figure 1. Spectrophotometric Curves of Molybdenum Blue Complex

A. Trichloroacetic acid extract of peanut meal, 0.029 mg. of phosphorus. Curve set off +10 units
B. Standard phosphorus solution, 0.030 mg. of phosphorus
C. Trichloroacetic acid extract of cottonseed meal, 0.031 mg. of phosphorus. Curve set off −10 units

Extraction of the molybdenum blue developed by the methods of Fontaine (*8*) and Gerritz (*9*) with 10 ml. of isobutyl alcohol produced solutions having single maxima at 780 mμ for the Fontaine method and at 800 mμ for the Gerritz method. For both procedures, the absorption maxima of the solutions prior to extraction with isobutyl alcohol were at 820 mμ. The shape of the spectrophotometric curves for each of the complexes extracted with isobutyl alcohol was similar to that of the curves for the unextracted complexes.

That the molybdenum blue developed by the shakeout method is very stable in the mixture of isobutyl and ethyl alcohol was shown when 0.005, 0.025, and 0.045 mg. of phosphorus were carried through the colorimetric procedure, placed in well-stoppered colorimeter tubes, and read in the Evelyn colorimeter (No. 720 filter) at once and at intervals up to 19 hours. The colors were found to increase slowly up to 40 minutes after development, and then remain constant for 19 hours. The transmission values of unknowns, checked from time to time after the solutions were allowed to stand overnight, have been found to be unchanged.

Table IV. Inorganic Phosphorus Content of Various Plant Materials on Dry Basis

Material	Total Phosphorus	Inorganic Phosphorus: 1-hour extraction	Inorganic Phosphorus: 24-hour extraction at 25° C.	Inorganic Phosphorus: 24-hour extraction at 5° C.
	%	%	%	%
Cottonseed meal, diethyl ether extracted	1.722	0.085	0.085	0.085
Cottonseed meal, Skellysolve B extracted	1.711	0.071	0.073	0.071
Peanut kernels, Skellysolve F extracted	0.849	0.081	0.082	0.079
Raw cotton fiber	0.028	0.015	0.015	0.015
Sweet potatoes, L-5	0.135	0.075	0.076	0.076
Jerusalem artichokes	0.385	0.083	0.085	0.085
U. S. 13 corn[a]	0.282	0.016	0.016	0.016
Milo[a]	0.274	0.016	0.018	0.017
Federation wheat[a]	0.377	0.018	0.019	0.019
Kharkof wheat[a]	0.445	0.017	....	....
Wheat straw[a]	0.165	0.118	0.122	0.119
Soybean meal, Skellysolve F extracted[b]	0.750	0.036	0.037	0.036
Phytin, crude, from peanuts[b]	14.31	0.070	....	....
Phytin, crude, from cottonseed[b]	13.84	0.061	....	....
Dialyzed peanut protein[b]	0.650	0.014	....	....
Dialyzed cottonseed protein[b]	1.164	0.013	....	....

[a] From Northern Regional Research Laboratory.
[b] Prepared by T. D. Fontaine.

PRECISION

Sixteen determinations made on the same sample of solvent-extracted peanut meal over a period of several months gave an average value of 0.0717% inorganic phosphorus with a standard deviation of ±0.0017%. Twenty-five determinations on another sample gave an average value of 0.0696% inorganic phosphorus with a standard deviation of ±0.0012%.

APPLICABILITY

The general applicability of the method is illustrated by Table IV, where the inorganic phosphorus content of a number of plant materials is reported. Total phosphorus values obtained by the reduced molybdate method of Gerritz (*9*) are also given.

ACKNOWLEDGMENT

The authors are indebted to R. T. O'Connor for the spectrophotometric curves, to E. B. Schuman for preparation of the figures, and to T. D. Fontaine for the preparation of the peanut and cottonseed meals and for a number of suggestions. The samples of glucose-1-phosphate and sodium β-glycerophosphate were obtained through the generosity of David M. Greenberg of the University of California Medical School and J. P. Remensnyder of the Heyden Chemical Corporation, respectively.

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Quantitative Determination of Ergosterol, Cholesterol, and 7-Dehydrocholesterol

Antimony Trichloride Method

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Methods for the quantitative determination of ergosterol, cholesterol, and 7-dehydrocholesterol are based upon the spectrophotometric measurement of the optical densities of the characteristic absorption maxima of the reaction products resulting from the treatment of the chloroform solutions of these sterols with an antimony trichloride–acetyl chloride reagent. The absorption spectra of the reaction products of the three sterols are distinctly different. By this method it is possible to differentiate between ergosterol and 7-dehydrocholesterol. This differentiation is not possible by the direct ultraviolet spectrophotometric method. A comparison has been made of results obtained by both the direct spectrophotometric and the antimony trichloride method in the determination of ergosterol in three different types of yeast and one mold extract.

THE most reliable method for the quantitative determination of ergosterol has been the spectrophotometric measurement of its ultraviolet absorption spectrum, with its characteristic absorption maxima at wave lengths of 262, 271, 281, and 293 millimicrons. However, as shown both by Hogness, Sidwell, and Zscheile (*6*) and Huber, Ewing, and Kriger (*7*), the ultraviolet absorption spectra of ergosterol and 7-dehydrocholesterol are so nearly identical that it is impossible to differentiate between these two compounds spectrophotometrically. Accurate spectrophotometric measurements in the ultraviolet region require that the sample be highly purified, since numerous organic compounds show either general or specific absorption in this region and would thus contribute to the absorption measurements. Furthermore, a spectrophotometer with ultraviolet accessories including a satisfactory ultraviolet light source is required for the determination of ergosterol by this method. A method requiring less expensive equipment and less labor in purification of sample than required by the direct ultraviolet method is desirable.

A number of colorimetric methods employing concentrated sulfuric acid (*1*, *9*, *12*), acetic anhydride (*2*, *5*, *10*, *16*), trichloroacetic acid (*14*), antimony trichloride (*5*, *8*, *13*), and various combinations of these reagents have been reported in the literature for the determination of ergosterol, 7-dehydrocholesterol, cholesterol, and other sterols. However, these methods as reported lack the desired specificity and sensitivity. Several of these methods were investigated by making absorption measurements of their respective colored reaction products by means of the Beckman quartz spectrophotometer Model DU. Some interesting observations, which were made on two of the above methods are reported here, since it is thought they may be of value to other investigators in this field.

In repeating the work of Petersen and Harvey (*12*) the authors have found that the colored solution produced by the treatment of ergosterol with 90% sulfuric acid has an absorption curve as shown in Figure 1 with two maxima. One maximum occurs at a wave length of 315 mμ with an $E^{1\%}_{1cm.}$ value of approximately 333 and another at 415 mμ with an $E^{1\%}_{1cm.}$ value of approximately 196. Spectrophotometric measurements show that the absorption maxima of this reaction product become stable 50 to 60 minutes after the addition of the reagent. These two maxima seem to have been overlooked by Petersen and Harvey, since they based their method of determination upon a measurement of the per cent transmission at a wave length of 550 mμ. While neither of the two observed maxima is very sharp or well defined, the $E^{1\%}_{1\ cm.}$ values are high and if measurements were made at either of these wave lengths instead of at 550 mμ the sensitivity and accuracy of the method might be greatly improved.

In a study of the method described by Goldhammer and Kuen (*4*), spectrophotometric measurements were made on the blue colored reaction product produced by the treatment of ergosterol with an antimony trichloride–acetic anhydride reagent in accordance with their method. Table I shows the absorption data of a chloroform solution of the ergosterol–antimony trichloride–acetic anhydride reaction product at various time intervals. The most prominent and stable maxima are the ones occurring at a wave length of 680 mμ with an $E^{1\%}_{1\ cm.}$ value of 43.6 and at 390 mμ with an $E^{1\%}_{1\ cm.}$ value of 55.0. The maximum at 390 mμ was found to be sharp and well defined but the extinction value is rather low for analytical purposes, and further it has not reached its maximum intensity at the end of 50 minutes. The 680 mμ maximum is also of low intensity.

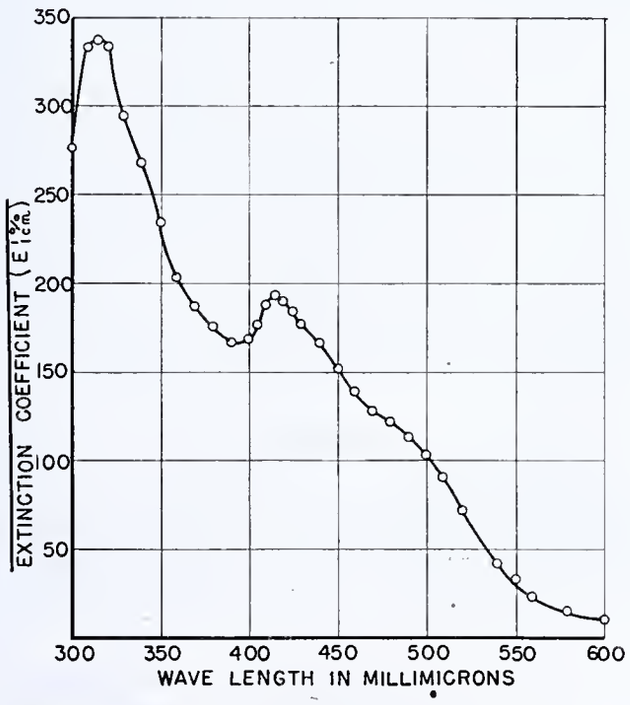

Figure 1. Absorption Spectrum of Ergosterol–Sulfuric Acid Reaction Product

The method for the determination of ergosterol which has proved most satisfactory for the authors' needs results from the treatment of ergosterol with an antimony trichloride–acetyl chloride reagent prepared according to Nield, Russell, and Zimmerli (*11*). Upon treatment of a chloroform solution of pure

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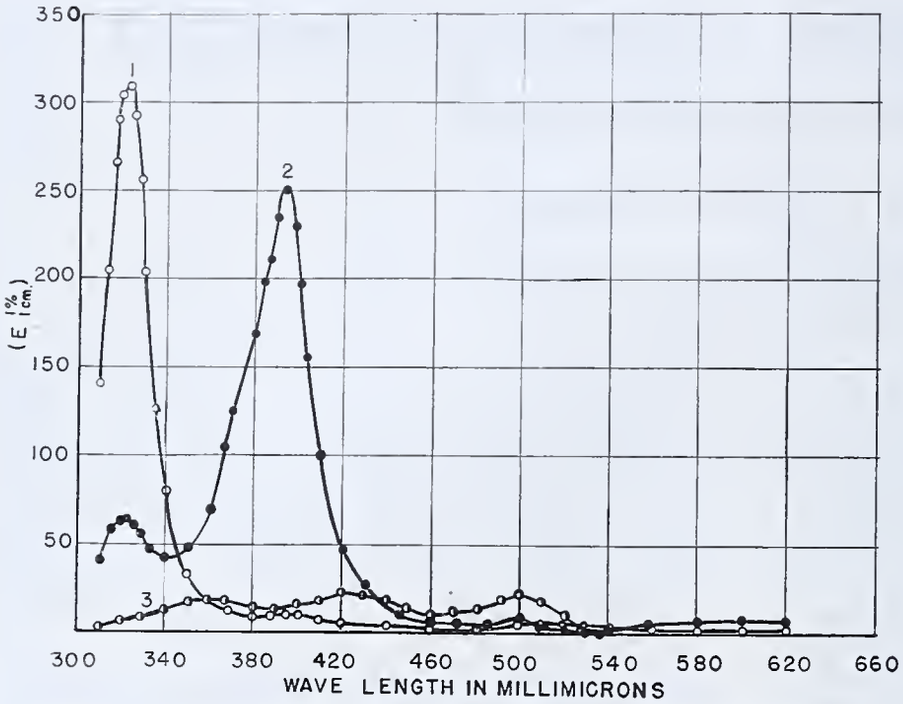


Figure 2. Absorption Spectra of Sterol–Antimony Trichloride–Acetyl Chloride Reaction Products

1. 7-Dehydrocholesterol–antimony trichloride–acetyl chloride reaction product. Measurements made 5 to 15 minutes after addition of reagent
2. Ergosterol–antimony trichloride–acetyl chloride reaction product. Measurements made 5 to 15 minutes after addition of reagent
3. Cholesterol–antimony trichloride–acetyl chloride reaction product. Measurements made 1 to 15 minutes after addition of reagent

ergosterol with this reagent a yellow color develops, which is stable at the end of 2 minutes. A reddish-yellow color is obtained on ergosterol samples which have not been purified. A chloroform solution of the colored compound was found to have an absorption spectrum as shown in curve 2 of Figure 2 with a prominent maximum at a wave length of 393 mμ with an $E^{1\%}_{1\text{ cm.}}$ value of 250 and two less intense maxima at 322 and 505 mμ. Upon investigation, the maximum at 393 mμ has proved to be sufficiently stable, sensitive, and specific for use as the basis of a quantitative method for the determination of ergosterol. This method has an advantage over the direct ultraviolet absorption method since it differentiates ergosterol from 7-dehydrocholesterol. The method has been used satisfactorily for the determination of ergosterol in such materials as yeast and plant extracts and various sterol mixtures. With the modifications described under "Absorption Spectra of Other Sterols" this method may also be adapted to the quantitative determination of cholesterol, 7-dehydrocholesterol, and other sterols.

REAGENTS

Chloroform. Merck's c.p. grade chloroform is purified in the manner described by Ewing *et al.* (*3*). The purified chloroform prepared in quantities of 1 liter has been found to be stable and satisfactory for use for 2 weeks and longer when stored over activated charcoal (Merck, U.S.P.) and anhydrous potassium carbonate. The chloroform stored in this manner is first shaken well and then filtered just prior to using. The usual qualitative tests for the presence of alcohol and phosgene are made to ensure their absence.

Antimony Trichloride–Acetyl Chloride Reagent. The antimony trichloride–acetyl chloride reagent is prepared according to the method of Nield, Russell, and Zimmerli (*11*). This reagent, containing 18 grams of antimony trichloride and 3 ml. of acetyl chloride per 100 ml. of chloroform (18% reagent), is used for the quantitative determination of both ergosterol and 7-dehydrocholesterol. For the determination of cholesterol a reagent containing 30 grams of antimony trichloride and 3 ml. of acetyl chloride per 100 ml. of chloroform (30% reagent) is prepared. In determining cholesterol the use of the 30% reagent was found to improve the reproducibility and sensitivity of the method. When pure dry chloroform and pure acetyl chloride are used very little difficulty is encountered with the reagent. It has been found to remain uncolored and satisfactory for use when stored for periods of 1 and 2 weeks in an amber bottle at room temperature.

EXPERIMENTAL PROCEDURE

Method. Solutions containing 0.005 to 0.05% ergosterol are prepared in purified chloroform. To 1 ml. of the ergosterol solution in a 50-ml. beaker, 10 ml. of the 18% antimony trichloride–acetyl chloride reagent are added. The optical density of the absorption maximum, which develops at 393 mμ, is read on a suitable spectrophotometer exactly 5 minutes after the addition of the reagent. A Beckman DU quartz spectrophotometer with 10.0-mm. Corex absorption cells and incandescent light source was used for all spectrophotometric measurements reported in this paper. The slit width is maintained at 0.068 to 0.072 mm. when taking readings at 393 mμ. The amount of ergosterol present in the solution is calculated from the $E^{1\%}_{1\text{ cm.}}$ value of 250 at 393 mμ for pure ergosterol.

Purification of Ergosterol and Determination of Its $E^{1\%}_{1\text{ cm.}}$ Value. In the preceding paper the extinction coefficients given at 322, 393, and 505 mμ were the results of measurements made upon commercial ergosterol. Since the ultraviolet absorption spectrum of this material conformed to the data reported in 1937 by Hogness, Sidwell, and Zscheile (*6*) it was considered to be reasonably pure ergosterol. However, when the ultraviolet absorption data of Huber, Ewing, and Kriger (*7*) were reported, it was realized that the commercial samples of ergosterol used for these observations were not 100% pure.

For this study commercial ergosterol was purified by recrystallization from acetone containing approximately 1% water. In the purification 4 to 5 grams of ergosterol were dissolved in 100 ml. of pure, acid-free acetone by heating on a 70° C. water bath. Three to 5 drops of distilled water were added to the hot solution with stirring. Upon slowly cooling to room temperature the ergosterol crystallized from the clear solution as fine, small needles. The crystals were filtered by suction and a portion dried in an Abderhalden vacuum dryer at 60° C. for 1 hour. The greater part of the crystals were redissolved in acetone and recrystallized. After each recrystallization a dried portion was used for determination of its melting point and $E^{1\%}_{1\text{ cm.}}$ values by the ultraviolet absorption and antimony trichloride methods. The data obtained are given in Table II.

These physical constants show an increase in purity of the ergosterol upon successive recrystallizations. For the eighth, ninth, and tenth recrystallizations the melting point remained constant and the ultraviolet and antimony trichloride values were constant within the limits of error for individual determinations. These findings are contrary to the statement of Huber *et al.* (*7*); "The highest values are obtained by recrystallizing

Table I. Extinction Coefficients of Absorption Maxima of Ergosterol–Antimony Trichloride–Acetic Anhydride Reaction Product at Various Time Intervals

Wave Length, Millimicrons	$E^{1\%}_{1\text{ cm.}}$ of Absorption Maxima		
	1 to 5 min.	15 to 30 min.	30 to 40 min.
390	4.1	34.0	55.0
520	23.0	No max.	No max.
580	No max.	24.0	No max.
680	20.0	43.6	43.6

Table II. Physical Constant Data for Ergosterol Following Successive Recrystallizations

Sample	Melting Point[a], ° C.	E (1%, 1 cm.)[b] Values at Following λ Ultraviolet 271 mμ	282 mμ	293 mμ	$SbCl_3$ 322 mμ	393 mμ	505 mμ
s received	155–164	242.8	255.3	148.2	66.7	212.1	33.3
ecrystallization							
1	162–165	269.3	285.2	162.3	69.1	239.8	20.3
2	164–166	269.7	284.1	161.8	61.3	241.3	17.8
3	164–166.5	270.4	285.9	161.4	59.9	246.1	14.9
4	164–166.5	276.0	291.6	163.1	59.2	248.2	11.4
5	164–166.5	275.0	288.1	165.4	61.3	245.3	10.2
6	164.5–167	274.5	288.3	163.6	70.0	252.0	13.2
7	165–167	276.7	289.6	167.1	64.9	249.7	12.1
8	166–167	276.1	289.1	164.9	63.6	250.4	13.1
9	166–167	275.5	287.6	164.2	60.2	249.0	13.6
10	166–167	276.9	290.8	166.5	63.5	252.2	13.1

[a] Melting points obtained with Anschütz thermometer and sealed melting oint tubes.
[b] Data represent single trials and consequently do not conform with verage values presented in Table III.

nce only the commercially available material". This divergence n results is undoubtedly due to the difference in solvents, since ese authors recrystallized their ergosterol from a benzene-thanol solvent. From Table II it is noted that as the ergosterol purified the antimony tricholoride maximum at 505 mμ de-reases and the maximum at 393 mμ increases, while the maxi-um at 322 mμ fluctuates although it shows a somewhat lower alue upon purification.

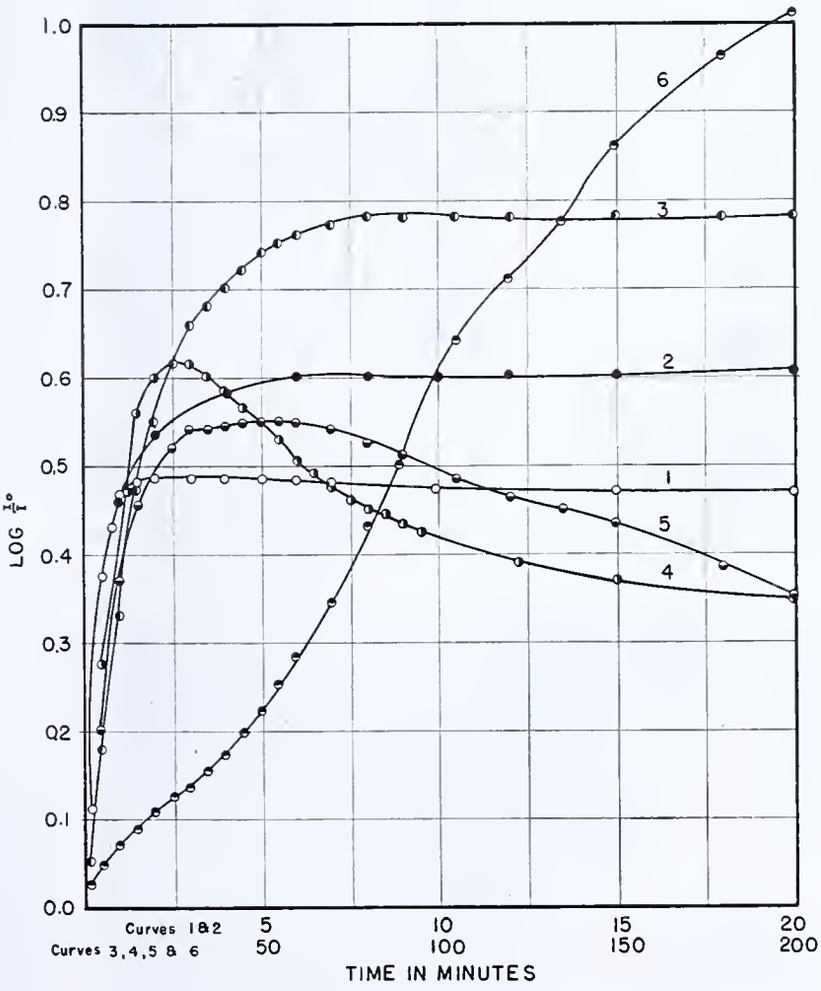
Over twenty individual determinations of the $E^{1\%}_{1\text{ cm.}}$ values t the 322, 393, and 505 mμ maxima were made on the purified rgosterol by the antimony trichloride method. The average $E^{1\%}_{1\text{ cm.}}$ value was found to be 62.9 at 322 mμ, 250.02 at 393 mμ, nd 12.4 at 505 mμ. The individual determinations of the $E^{1\%}_{1\text{ cm.}}$ alues at 393 mμ showed a maximum deviation of ±2.0% from he average. For optical density (log I_0/I) readings f 0.20 to 1.00, the ergosterol–antimony trichloride–cetyl chloride reaction product was found to obey eer's law.

Time Rate of the Reaction. The absorption naximum of the ergosterol–antimony trichloride–acetyl hloride reaction product at 393 mμ reaches its maxi-um intensity in a little less than 2 minutes after the ddition of the reagent and remains stable for 6 to 7 inutes. The rate of the reaction is shown graphically n curve 1 of Figure 3, in which the optical density (log $_0/I$) at 393 mμ is plotted against time. The optical density readings become steady after the addition of the reagent and remain constant for 6 minutes. For this reason the procedure standardized upon in the method for determining ergosterol has been to make the spectrophotometric measurements exactly 5 minutes after the addition of the antimony trichloride–acetyl chloride reagent.

Effect of Temperature. Ergosterol determinations were made at 20°, 25.5°, and 30° C, which cover the range of ordinary laboratory temperatures. The results obtained showed no measurable variation.

Effect of Solvent Purity. The reproducibility of the reaction between ergosterol and the antimony trichloride reagent as determined by spectrophotometric measurements is very sensitive to the presence of traces of moisture and polar solvents, which if present may cause the results to vary as much as 10%. To ensure reproducible results the reagents must be carefully purified and the sample dried in a stream of warm carbon dioxide (80° to 90° C.).

Absorption Spectra of Other Sterols. Figure 2 shows the absorption spectra of the antimony trichloride–acetyl chloride reaction product with ergosterol, cholesterol, and 7-dehydrocholesterol, using the 18% reagent. All measurements were made during the 1- to 15-minute period following the addition of the antimony trichloride reagent. From these curves it is seen that the reaction products of 7-dehydrocholesterol and ergosterol have absorption spectra which are distinctly different, making it possible to differentiate the two clearly and also to determine them individually in the presence of each other. Except for the method recently reported by Sobel, Mayer, and Kramer (*15*) for the differentiation of ergosterol and 7-dehydrocholesterol by treating the sterols with glycerol dichlorohydrin in the presence of acetyl chloride, this is the only method known to the authors for the differentiation and determination of ergosterol



1. Optical density measurements with time of absorption maximum at 393 mμ of ergosterol reaction product. Concentration of ergosterol 0.002072%
2. Optical density measurements with time of absorption maximum at 322 mμ of 7-dehydrocholesterol reaction product. Concentration of 7-dehydrocholesterol 0.001875%
3. Optical density measurements with time of absorption maximum at 320 mμ of cholesterol reaction product. Concentration of cholesterol 0.0069%
4. Same as 3 except for absorption maximum at 360 mμ of cholesterol reaction product
5. Same as 3 except for absorption maximum at 420 mμ of cholesterol reaction product
6. Same as 3 except for absorption maximum at 500 mμ of cholesterol reaction product

Figure 3. Time Reaction Curves

and 7-dehydrocholesterol, when present together. The absorption curve for cholesterol is of relatively low intensity. Consequently the presence of cholesterol would not interfere appreciably with the determination of either ergosterol or 7-dehydrocholesterol. The four absorption maxima of the cholesterol reaction product develop much more slowly and irregularly than the maxima for ergosterol and 7-dehydrocholesterol.

Figure 3 shows the time rate of the development of the various absorption maxima of the cholesterol, ergosterol, and 7-dehydrocholesterol reaction products, using the 18% antimony trichloride reagent. In the case of ergosterol the absorption maximum at 393 mμ is stable during 2 to 8 minutes after the addition of the reagent, and in the case of 7-dehydrocholesterol the absorption maximum at 322 mμ is stable during 6 to 15 minutes after addition of the reagent. The maximum for cholesterol at 360 mμ develops strongly during the first 20 to 25 minutes and then decreases in intensity. Of the four maxima, the one at 360 mμ was found to be the most satisfactory for the determination of cholesterol from the standpoint of sensitivity, reproducibility, time for development of maximum intensity, and adherence to Beer's law. The results obtained with the 420, 500, and 320 mμ maxima show considerable variation and the reproducibility is poor, with either the 18% or 30% reagent. A summary of the absorption maxima, corresponding $E^{1\%}_{1\,cm.}$ values, optimum concentration range, and time for development of stable absorption maxima for the three sterol antimony trichloride–acetyl chloride reaction products is given in Table III. The data for cholesterol with both the 30% and 18% reagents are given.

Table III. Absorption Data for Ergosterol, Cholesterol, and 7-Dehydrocholesterol–Antimony Trichloride–Acetyl Chloride Reaction Products

Sterol	λ of Maximum mμ	$E^{1\%}_{1\,cm.}$	Range of Optimum Concentration (Final) %	Reading Time[a] *Min.*
Ergosterol	322	62.9	...	...
	393	250.0	0.0005–0.005	5
	505	12.4	...	...
7-Dehydrocholesterol	322	320.0	0.0003–0.003	8
	390	10.0	...	...
	510	6.7	...	...
Cholesterol (with 18% reagent)	320	111.7	...	90
	360	99.9	0.001 –0.010	25
	420	63.9	...	40
	500	87.5	...	120
Cholesterol (with 30% reagent)	320	125.9	...	90
	360	168.8	0.0006–0.006	20
	420	53.8	...	40
	500	40.3	...	120

[a] Time at which optical density readings were taken for calculation of $E^{1\%}_{1\,cm.}$ values recorded in column 3. With exception of 120-minute readings for cholesterol at 500 mμ, times stated are those for development of maximum optical density readings at various absorption maxima.

By rewording the method as written for ergosterol under "Experimental Procedure" so as to read "solution containing 0.003 to 0.03% 7-dehydrocholesterol", and by taking the optical density readings at 322 mμ exactly 8 minutes after the addition of the reagent, the method may be used for the determination of 7-dehydrocholesterol. The amount of 7-dehydrocholesterol present in the solution is calculated from the $E^{1\%}_{1\,cm.}$ value of 320.0 at 322 mμ for pure 7-dehydrocholesterol. The ultraviolet absorption spectrum indicated the 7-dehydrocholesterol used in this work to be 100% pure.

The reproducibility of results and adherence to Beer's law were found to be very satisfactory. The same precautions observed with the antimony trichloride reagent for the ergosterol determination should also be observed for the 7-dehydrocholesterol and cholesterol determinations. Further discussion of this method and its application to the determination of 7-dehydrocholesterol will be given by the authors in a later paper.

Table IV. Comparison of Ergosterol Determinations in Yeast and Mold Extracts by Direct Spectrophotometric and Antimony Trichloride Methods

Sample	Calculated from Spectrophotometric Measurements 271 mμ	282 mμ	293 mμ	Antimony Trichloride Method
	Milligrams per gram			
Yeast 1	1.17	1.05	1.24	1.03
Yeast 2	2.78	2.63	2.87	2.29
Yeast 3	2.94	2.79	3.06	52.41
Mold 1	6.18	6.12	8.37	4.80

For the determination of cholesterol, it was found preferable to use a reagent containing 30 grams of antimony trichloride and 3 ml. of acetyl chloride per 100 ml. of chloroform. In determining cholesterol, 10 ml. of the 30% reagent are added to 1 ml of a chloroform solution containing 0.006 to 0.06% cholesterol. The optical density reading at 360 mμ is taken at its maximum value, which is reached during the 20- to 25-minute period following the addition of the reagent. The amount of cholesterol is calculated from the $E^{1\%}_{1\,cm.}$ value at 360 mμ for pure cholesterol. The average $E^{1\%}_{1\,cm.}$ value for pure cholesterol at 360 mμ was found to be 168.8 with a maximum deviation of ± 5.0%.

DISCUSSION

The antimony trichloride method here described has been applied to the determination of ergosterol in one plant and three yeast extracts. The ultraviolet absorption data were also obtained. A comparison of the results by the two methods is tabulated in Table IV. In each case the results by the antimony trichloride method are lower than those obtained by a direct measurement of the ultraviolet absorption spectrum. This may be attributed to the fact that the direct spectrophotometric method is more liable to high results due to the presence of impurities. The variation in per cent concentration of ergosterol calculated at the maxima 271, 282, and 293 mμ also indicates the presence of impurities. It is likely that the interfering substances are contributing to the measured density values.

A thorough investigation of the application of the antimony trichloride method to natural materials has not yet been made; however, the few results presented in Table IV are indicative of its possibilities. In determining the amount of ergosterol in impure samples these data indicate the advisability of supplementing the ultraviolet absorption measurements with the antimony trichloride values in order to make a more complete evaluation of the sample.

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New Method for Determination of Manganese

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A new method for determination of manganese is based on the potentiometric titration of manganous ion with permanganate ion in neutral pyrophosphate solution. The method is applicable without any separations to a wide variety of manganiferous metallurgical products, and it yields very accurate results.

THIS paper presents a new method for the determination of manganese based on the titration of manganous ion with permanganate ion in neutral pyrophosphate solution, the +2 manganese being oxidized, and the permanganate ion reduced, to a pyrophosphate complex of the +3 state. The stoichiometry of the reaction may be represented by

$$4\,Mn^{++} + MnO_4^- + 8\,H^+ + 15\,H_2P_2O_7^{--} = 5\,Mn(H_2P_2O_7)_2^{---} + 4\,H_2O \quad (1)$$

Since the intense reddish violet color of the pyrophosphate complex of +3 manganese precludes the use of a color indicator, the titration is performed potentiometrically.

In solutions of the proper pH the titration reaction is stoichiometrically exact, and hence the titrant permanganate solution can be standardized by any of the usual procedures. The method is at least as accurate, and even less subject to interferences, then the bismuthate method, which is generally conceded to be the most reliable of existing methods for manganese (*1, 3, 4, 6, 7, 8*). In particular, even large amounts of chloride, cobalt, and chromium do not interfere with the present method as they do in most other procedures, including the bismuthate method. Practically all other elements commonly associated with manganese, such as iron, nickel, copper, molybdenum, tungsten, and uranium, are also innocuous. The method has been applied successfully, without any preliminary separations, to a wide variety of manganiferous materials, including pyrolusite ore, ferromanganese, spiegeleisen, copper-base alloys, and alloy steels, with an accuracy equal to that obtainable by the bismuthate method. In spite of the fact that the titration is performed potentiometrically, the time required for an analysis by the present method is no greater than in the bismuthate method, because no filtrations are involved.

The development of the present method was facilitated by information gleaned from the recent excellent studies by Watters and Kolthoff (*9, 10*) of the properties of the pyrophosphate complexes of +3 manganese. These authors developed polarographic and amperometric titration procedures for the determination of manganese after oxidation to the +3 manganese pyrophosphate complex by excess lead dioxide. Watters (*9*) also described a volumetric method based on oxidation with lead dioxide, removal of the excess of the latter by filtration, and subsequent titration of the +3 complex with standard ferrous solution with diphenylbenzidine indicator. These methods are subject to interference by chloride ion, chromium, vanadium, and other elements which are oxidized by lead dioxide.

GENERAL EXPERIMENTAL TECHNIQUE

Standard solutions of manganous ion in dilute sulfuric acid were prepared in two ways; some were prepared from manganous oxalate dihydrate, which had been synthesized from recrystallized potassium permanganate and oxalic acid by the method of Coltman (*2*) and air-dried, and others were prepared from Bureau of Standards manganese ore No. 25b. Weighed portions of the manganous oxalate dihydrate were decomposed by digestion with concentrated sulfuric acid in a Kjeldahl flask, and diluted to volume in calibrated volumetric flasks. The purity of the salt was checked by determining its oxalate content by the usual permanganimetric procedure, with a permanganate solution that had been standardized against sodium oxalate under exactly the same conditions. The average percentage of oxalate ion found in ten determinations was 49.21 ± 0.06, which agrees to within 1 part per 1000 with the theoretical value 49.17. Standard solutions were prepared from the manganese ore by decomposing weighed samples (dried at 120° C.) in a mixture of hydrochloric and sulfuric acids in a Kjeldahl flask, boiling down to fumes of sulfur trioxide, and finally diluting to volume.

Standard 0.02 molar potassium permanganate solutions were prepared and standardized against pure sodium oxalate by the usual procedure (*4, 6*). Some of the solutions were also standardized against anhydrous potassium ferrocyanide by potentiometric titration in 2 *N* sulfuric acid at room temperature according to the method described by Kolthoff (*5*). The potassium ferrocyanide had been recrystallized twice and dehydrated by heating to constant weight at 110° C. The titer obtained with potassium ferrocyanide agreed very well with that obtained with sodium oxalate; in a typical case a solution was found to be 0.01857 molar against sodium oxalate and 0.01858 molar against potassium ferrocyanide. Standard 0.002 molar permanganate solutions were prepared by appropriate dilution of the 0.02 molar solutions with high-quality distilled water; these dilute solutions were prepared immediately before use. In the titration of manganese by the procedure described herein the permanganate ion undergoes only a 4-electron reduction to the +3 state, and hence a 0.02 molar solution is 0.08 *N*, and 1 cc. corresponds to 4.394 mg. of manganese.

Weights and volumetric equipment were calibrated with care, and temperature corrections were applied in measuring the solutions.

All reagents were of analytical reagent quality. Sodium pyrophosphate ($Na_4P_2O_7 . 10H_2O$) was used in the form of freshly prepared solutions saturated at room temperature (ca. 12 grams per 100 cc. or 0.3 *M*). Although the hydrolysis of the pyrophosphate ion is slow in cold neutral or alkaline solutions (*11*), it is advisable not to use solutions that are more than 2 or 3 weeks old. Samples of sodium pyrophosphate (analytical reagent grade) from three different manufacturers were used, and most of them were satisfactory. However, with some of the samples erratic potentials were observed during the titrations of manganese under the conditions hereinafter described, and 0.2 to 0.3 cc. too much 0.02 molar permanganate was used. This atypical behavior was apparently caused by some impurity in the samples, because it disappeared when the salt was recrystallized. Hence, it is advisable to test each new lot of salt by using it in the titration of a known amount of manganese, and if steady potentials are not established rapidly (as is typical of this titration) the salt should be recrystallized.

All reagents used must be completely free from reducing substances. In particular the dilute sodium hydroxide solution (ca. 5 *N*) which may be required to adjust the pH of the solutions prior to titration should be tested by adding a drop of 0.02 molar permanganate to a 10-cc. sample. No green color due to manganate ion should develop. It is advisable to prepare the sodium hydroxide solution freshly as needed; solutions which have stood in rubber-stoppered bottles invariably become contaminated by reducing substances, and these cause erratic potential readings during the titration and consume permanganate.

The titrations were performed in ordinary beakers, with the solutions stirred by a motor-driven glass stirrer. Bright platinum indicator electrodes were used in combination with a saturated calomel reference electrode. The e.m.f. was measured with an ordinary potentiometer in most of the titrations. Since the potential break at the end point is very large, simple potentiometric equipment, reading to a few millivolts, suffices. A number of titrations were made rapidly and accurately with a simple high-resistance voltmeter, consisting of a Rubicon box-type galvanometer in series with a 300,000-ohm resistance and a tapping key; this instrument had a range of 1 volt and permitted readings to ±0.01 volt.

CHARACTERISTICS OF THE TITRATION

Typical potentiometric titration curves of +2 manganese with permanganate in pyrophosphate solutions of various pH values are shown in Figure 1. In all cases the amount of manganese titrated was 83.6 mg. (1.522 millimoles) as manganous sulfate,

the initial volume was 180 cc., and the total concentration of sodium pyrophosphate was approximately 0.27 *M*. The pH was adjusted by addition of dilute sulfuric acid or sodium hydroxide, and measured with a glass electrode. The titrations were made at room temperature. Air was removed from the solutions with nitrogen because it was thought that some air oxidation of the manganese might occur, but later it was established that no appreciable air oxidation takes place if the pH of the solution is 8 or smaller; titration curves obtained in the presence of air are identical in all respects with those obtained in a nitrogen atmosphere.

Although the titration can be performed successfully at all pH values between about 1 and 8, the potential break at the equivalence point is maximal when the pH is between 6 and 7. When the pH is decreased below about 6 the magnitude of the potential break decreases markedly, and at pH values above about 8 no break at all is observed at the point corresponding to oxidation of the manganese to the +3 state. At all pH values below 8 in these titrations the end point determined from the maximum of $\Delta E/\Delta V$ was at 20.50 ± 0.02 cc., which agrees exactly with the theoretical volume of 0.01857 molar permanganate required to oxidize 1.522 millimoles of manganese to the +3 state.

The diminishing potential break with decreasing pH results from the fact that the potential of the manganous-manganic couple increases more rapidly than that of the permanganate-manganic couple with decreasing pH. The large effect of pH on the potential of the manganous-manganic couple in these solutions reflects a change in the predominating species of pyrophosphate ion, and hence in the manganese complex ions, with changing pH. From the dissociation constants of pyrophosphoric acid (1.4×10^{-1}, 1.1×10^{-2}, 2.1×10^{-7}, and 4.0×10^{-10}), the predominant species is $H_2P_2O_7^{--}$ at a pH between 2.0 and 6.7, $HP_2O_7^{---}$ between pH 6.7 and 9.4, and $P_2O_7^{----}$ above pH 9.4. Watters and Kolthoff (*9*, *10*) have presented evidence that the manganic complex has the formula $Mn(H_2P_2O_7)_3^{---}$ at a pH between about 2 and 5, but at a pH between 6 and 8 the complex probably contains the mono- rather than the dihydrogen pyrophosphate ion.

At a pH much above 8 the pyrophosphate complex of +3 manganese is unstable and disproportionates into hydrous manganese dioxide and +2 manganese. This disproportionation is reversible; when a solution which has been titrated to the equivalence point at a pH between 6 and 7 is made strongly alkaline with sodium hydroxide hydrous manganese dioxide precipitates, but when the mixture is acidified the precipitate dissolves and the solution again assumes the characteristic reddish violet color of the +3 manganese complex.

The effect of pH on the standard potential of the manganous-manganic couple in pyrophosphate solutions of pH 0 to 9 has been studied by Watters (*9*), and the sections of the curves before the equivalence points in Figure 1 agree with what one would predict from his data. No previous data are available for the potential of the permanganate-manganic couple in pyrophosphate solutions as a function of pH; the curves in Figure 1 indicate that the standard potential of this couple is in the neighborhood of 1.25 volts *vs.* the saturated calomel electrode at pH = 0, and hence very close to the potential of the permanganate-manganous ion couple.

The optimum pH for the titration is between 6 and 7. In practice, a 25- or 50-cc. sample of the acidic manganese solution to be analyzed is added to 150 to 300 cc. of saturated sodium pyrophosphate solution (ca. 0.3 *M*), and the pH is adjusted to a value between 6 and 7 by addition of either dilute sulfuric acid or sodium hydroxide. If the solution is colorless, or nearly so, the proper pH is easily attained by adding bromothymol blue or chlorophenol red indicator and neutralizing to the intermediate yellowish green of the former or to an orange with the latter. With highly colored solutions, such as those encountered in the analysis of nickel or chromium steels, the pH adjustment is conveniently made with the aid of indicator test papers. Since the solution is well buffered the pH adjustment is easily accomplished.

In titrations at a pH of 6 to 7 the potential of the platinum indicator electrode becomes constant quickly after each addition of permanganate solution, and a complete titration can be performed in 15 to 20 minutes. Since steady potential readings are attained quickly, the technique of titrating directly to the equivalence point potential can be applied advantageously with a consequent shortening of the titration time to 10 minutes or less. With relatively pure manganese solutions, and a concentration of sodium pyrophosphate between 0.2 and 0.3 *M*, the equivalence point potential at a pH between 6 and 7 is +0.47 ± 0.02 volt *vs.* the saturated calomel electrode. In the presence of very large amounts of other metals a slightly lower value is usually found.

At a pH of 6 to 7 the magnitude of the potential change at the equivalence point—i.e., the maximal value of $\Delta E/\Delta V$—is between 100 and 200 mv. per 0.1 cc. of permanganate solution, and it is virtually independent of the amount of manganese titrated, the concentration of the permanganate solution, and the volume of the solution. This is the expected behavior for a homogeneous titration reaction.

The effect of the concentration of pyrophosphate was studied by titrating 100 mg. of manganese at a pH of 6 in various concentrations of sodium pyrophosphate from 0.04 to 0.3 *M* in a volume of 250 cc. The titration curves obtained with concentrations of sodium pyrophosphate between 0.15 and 0.3 *M* displayed virtually identical characteristics. With 0.04 *M* sodium pyrophosphate the potentials at all points in the titration were 0.05 to 0.1 volt more positive, and the potential break at the equivalence point was somewhat smaller (120 instead of 180 mv. per 0.1 cc.) than with the larger concentrations of pyrophosphate. With 100 mg. of manganese in 250 cc. a white precipitate of

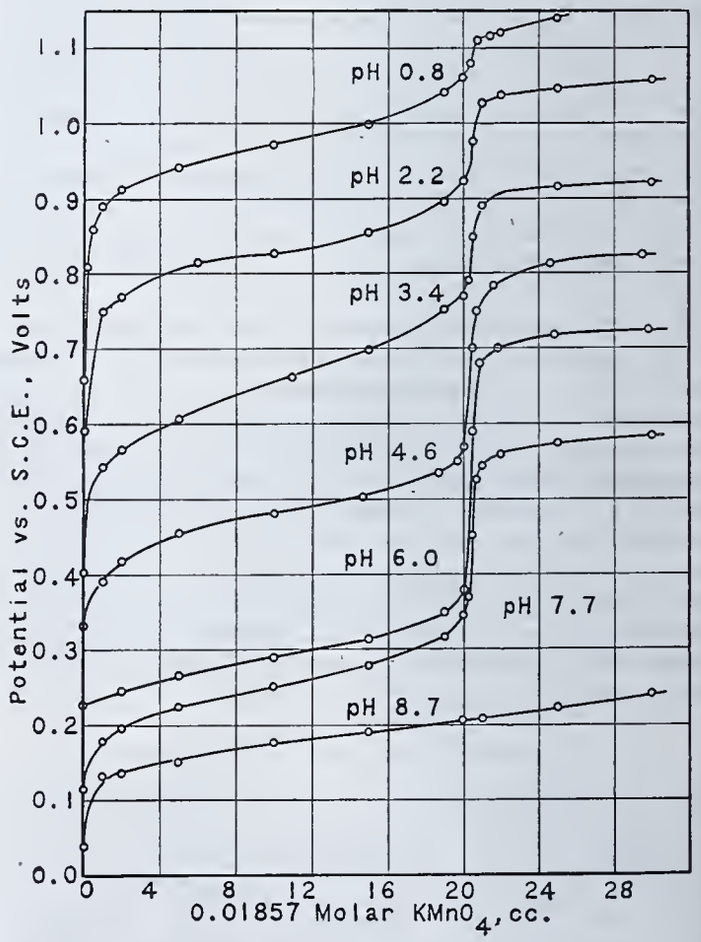

Figure 1. Influence of pH on Potentiometric Titration Curve of Manganous Ion with Permanganate Ion in Pyrophosphate Solution

nanganous pyrophosphate forms if the concentration of sodium pyrophosphate is smaller than about 0.2 *M*. This precipitate dissolves completely before the equivalence point is reached, and it does not interfere with the titration.

A small blank correction was found with some samples of sodium pyrophosphate. This was determined by titrating 200-cc. samples of saturated sodium pyrophosphate alone with 0.002 molar permanganate after adjusting the pH to 6 with dilute sulfuric acid. In some cases the potential jumped sharply from about +0.2 to above +0.45 volt after adding only one drop of the 0.002 molar permanganate solution, indicating a negligibly small blank. With other samples of sodium pyrophosphate 0.10 to 0.15 cc. of 0.002 molar permanganate were required to obtain the characteristic potential break. Since at worst the blank amounts to only about 0.05 mg. of manganese, and usually is considerably less, it is small enough to be neglected when the amount of manganese titrated is greater than about 50 mg. With smaller amounts of manganese the appropriate correction should be applied.

The titration has been applied to amounts of manganese from a few milligrams up to 200 mg. in a volume of 150 to 500 cc. With relatively pure manganese solutions the accuracy of the titration appears to be limited only by the accuracy of the volumetric measurements.

STOICHIOMETRICAL EXACTNESS OF THE TITRATION REACTION

The exactness of the stoichiometry of the titration reaction was verified without reference to any standard substance in the following manner:

Samples of about 100 grams of a 0.02 molar permanganate solution were weighed, transferred to a Kjeldahl flask, acidified with 15 cc. of 4 *N* sulfuric acid, and reduced to manganous ion by dropwise addition of a fresh saturated solution of sodium sulfite. The slight excess of sulfur dioxide was expelled by boiling, after which the solution was cooled, transferred to the titration beaker, diluted to about 250 cc., and treated with 25 grams of recrystallized sodium pyrophosphate. The pH of the resulting solution was approximately 6. A sample of the original permanganate solution, 0.2 to 0.5% smaller than the theoretical one fourth of the sample that was reduced, was weighed and added to the solution. The titrations were finally completed with a very dilute permanganate solution (25 grams of the original solution diluted to 1 liter) from an ordinary buret.

The results of four such experiments are shown in Table I. The average ratio of the solution weights agrees exactly with the theoretical value of 4 predicted by Equation 1, and the average deviation from the mean is only ±0.05%.

Table I. Stoichiometry of Titration Reaction

$KMnO_4$ Solution Reduced	$KMnO_4$ Solution Required to Titrate	Ratio
Grams	*Grams*	
99.747	24.917	4.0032
99.965	25.004	3.9980
99.854	24.966	3.9996
99.843	24.969	3.9987
		Av. 4.000 ± 0.002

TITRATION OF MANGANESE IN PRESENCE OF OTHER ELEMENTS

From the conditions extant during the titration, and the fortunate circumstance that most metals form complexes with pyrophosphate that are soluble at a pH of 6 to 7, it was expected that very few elements would interfere with the determination of manganese. This has been substantiated by the series of experiments shown in Table II, in which a small amount of manganese (10 mg.) was titrated in the presence of large added amounts of those elements which more or less commonly accompany manganese. These experiments simulate conditions encountered in the determination of only 0.5 to 2% manganese in various metallurgical products and alloy steels.

Table II. Determination of Manganese in Presence of Other Elements

[10.62 mg. of manganese taken in all cases. Titrated with 0.002 molar permanganate (standardized against sodium oxalate) in 0.25 to 0.30 *M* sodium pyrophosphate at pH 6 to 7.]

No.	Addition	Volume	Mn Found	Error
	Mg.	*Cc.*	*Mg.*	%
1	None	175	10.64	+0.2
			10.63	+0.1
2	9000 KCl	175	10.62	0
			10.63	+0.1
3	1000 Fe^{+++}, 250 each Cu^{++}, Cr^{+++}, Ni^{++}, Co^{++}	400	10.66	+0.4
			10.56	−0.6
			10.58	−0.4
4	1000 Fe^{+++}, 100 each Mo^{++++++}, W^{++++++}, U^{++++++}	250	10.58	−0.4
			10.62	0
			10.62	0
5	250 Zn, 125 Al, and 50 each Mg, Cd	350	10.61	−0.1
			10.60	−0.2
			10.61	−0.1
6	1 V^{+++++}	180	10.61	−0.1
			10 63	+0.1
7	2.5 V^{++++}	180	10.60[a]	−0.2
			10.62	0
8	100 V^{+++++}	180	10.6[b]	...
9	800 Fe^{+++}, 400 V^{+++++}	300	10.6[b]	...
			10.6	...

[a] Corrected for volume of permanganate required to oxidize vanadyl ion.
[b] Titrated at pH 3 to 3.5; see text.

Since small amounts of manganese were present as an impurity in some of the salts used, particularly those of iron, cobalt, and nickel, blank determinations were run on each mixture and the appropriate correction was applied. This blank correction varied from a few hundredths up to 0.68 mg., depending on the amount and kind of metal salt.

Large amounts of chloride, ferric iron, +2 cobalt, copper, nickel, +3 chromium, +6 molybdenum, +6 tungsten, +6 uranium, zinc, aluminum, magnesium, and cadmium, have no significant effect. Nitrate, sulfate, and perchlorate ions are also harmless. The noninterference of chloride, cobalt, and chromium is a unique advantage of the present method over the classical bismuthate procedure.

Under the conditions stated, clear solutions were obtained in every case. With much larger quantities of certain metals, notably magnesium, cadmium, and aluminum, precipitates formed. Since some of these precipitates coprecipitate manganese, determinations should not be attempted in their presence.

Oxides of nitrogen interfere, and cause high results, because they are titrated by the permanganate. Hence when nitric acid is used to dissolve samples, the resulting solutions must not only be boiled thoroughly but a small amount of urea or sulfamic acid should be added to the acid solutions to remove the last traces of nitric oxide before adding the sodium pyrophosphate.

Of the elements likely to be associated with manganese, vanadium is the only one which causes difficulties, and then only when its amount is equal to or larger than the amount of manganese. When it is present originally in the +4 state vanadium is titrated to the +5 state along with the manganese. If, as is usually the case in steel analyses, the amount of vanadium is considerably less than that of the manganese the appropriate correction can be applied; an example is furnished by experiment 7 in Table II. When present in the +5 state in a pyrophosphate solution of pH 6 to 7 vanadium partially oxidizes the manganese, and is reduced to the +4 state. This causes no particular difficulty, and no error provided that the amount of vanadium is small (less than one fifth the amount of manganese), because the +4 vanadium so produced is subsequently reoxidized by the titrant permanganate solution (see Table II, experiment 6). However, the reaction between the +4 vanadium and permanganate ion is rather slow and with large amounts of vanadium a long time is required to obtain constant potentials, so that only small amounts of vanadium can be tolerated.

By working at a lower pH, and thus raising the potential of the manganous-manganic couple to a greater extent than that of the vanadyl-vanadate couple, the oxidation of manganese by +5 vanadium can be prevented. Under this condition it is possible to determine 10 mg. of manganese in the presence of as much

Table III. Determination of Manganese in Bureau of Standards Samples

(Permanganate solutions standardized against sodium oxalate)

Sample and Bureau of Standards Certificate Values	Mn Found, %
Manganese ore 25b Mn 58.35	58.35 58.37 58.33 58.29 58.35 58.38
	Av. 58.35 ± 0.02
Ferromanganese 68a Mn 80.07, C 6.83, Si 0.81, P 0.29, S 0.01	80.04 80.15 80.19 80.08
	Av. 80.12 ± 0.06
Spiegeleisen 66 Mn 19.93, Fe 73.45, C 4.05, Si 2.22, P 0.07, S 0.016, Ni 0.015, V 0.012, Ti 0.20, Cu 0.019, Cr 0.009, Co 0.01, Mo 0.005	19.97 20.01 20.00 20.00
	Av. 20.00 ± 0.01
Manganese rail steel 100 Mn 1.38, C 0.617, Si 0.19, P 0.02, S 0.02, Copper 0.12, Ni 0.15, Cr 0.18, V 0.011, Mo 0.005	1.385 1.381 1.392
	Av. 1.386 ± 0.004
Manganese bronze 62b Mn 1.28, Cu 57.40, Zn 37.97, Sn 0.97, Pb 0.27, Al 0.97, Fe 0.81, Ni 0.27, Si 0.05	1.277 1.274 1.273
	Av. 1.275 ± 0.002
Copper-nickel-chromium cast iron 115 Mn 1.01, Cu 6.44, Ni 15.89, Cr 2.17, V 0.009, Ti 0.021, Co 0.08, Si 1.60, C 2.42, P 0.113, S 0.032, Mo 0.002, and As 0.007	0.989 0.996 1.001 1.001
	Av. 0.997 ± 0.004
Chromium-vanadium steel 30d Mn 0.786, V 0.190, Cr 1.15, Cu 0.092, Ni 0.150, Mo 0.034, C 0.363, Si 0.286, P 0.031, and S 0.031	0.796 0.796 0.796
	Av. 0.796
Chromium-nickel steel 101b Mn 0.597, Ni 8.99, Cr 18.49, V 0.049, Co 0.078, Mo 0.078, Cb 0.062, Sn 0.012, Cu 0.168, C 0.069, Si 0.483, S 0.025, and P 0.017	0.60 0.63 0.61 0.60 0.66
	Av. 0.62 ± 0.02
Chromium-molybdenum steel 135 Mn 0.458, Cr 5.15, Mo 0.575, Cu 0.076, Ni 0.083, V 0.010, C 0.094, Si 0.383, P 0.017, and S 0.010	0.456 0.467 0.464
	Av. 0.462 ± 0.004

as 400 mg. of +5 vanadium, with an accuracy of about 1% (experiments 8 and 9 in Table II).

DETERMINATION OF MANGANESE IN METALLURGICAL PRODUCTS AND STEELS

To test the reliability of the present method under practical conditions, and compare its performance with other methods, a variety of samples were analyzed (Table III).

In general, a suitable weight of sample was decomposed in a Kjeldahl flask with an appropriate acid, and, after diluting and boiling thoroughly to remove gaseous reaction products, the resulting solutions were cooled and made up to volume in calibrated volumetric flasks without removing silica or the small amounts of carbon that usually remained. Whenever nitric acid was used to dissolve the samples 1 gram of urea or sulfamic acid per 250 cc. was added before finally diluting to volume, to ensure complete absence of nitric oxide. Aliquot portions (usually 50 cc.) of these solutions were pipetted into 200 to 300 cc. of saturated sodium pyrophosphate solution, and after adjusting the pH to a value between 6 and 7, the titrations were performed as already described. The titrant permanganate solutions (0.02 molar for samples 25b, 68a, and 66, and 0.002 molar for the others) were standardized against sodium oxalate.

Manganese Ore 25b. Six- to 8-gram portions of this sample, which consists mainly of pyrolusite, were dried at 120° C. and decomposed with a mixture of 100 cc. of 1 to 1 hydrochloric acid and 25 cc. of concentrated sulfuric acid, and finally diluted to 1 liter. Aliquots containing from 80 to 160 mg. of manganese were titrated. The time required for the analysis of pyrolusite ores by the present method is considerably less than that required in the bismuthate method, chiefly because hydrochloric acid can be used to dissolve the sample and no filtration is required.

Ferromanganese 68a and Spiegeleisen 66. One-gram samples of the ferromanganese and 3-gram samples of the spiegeleisen were decomposed by boiling with 50 cc. of 1 to 1 nitric acid. The solutions were finally diluted to 250 cc., and 50-cc. aliquots (120 to 160 mg. of manganese), were titrated.

Manganese Rail Steel 100 and Manganese Bronze 62b. Five-gram samples were dissolved in 50 to 100 cc. of 1 to 1 nitric acid. The solutions were diluted to 250 cc., and 50-cc. aliquots, corresponding to 12 to 14 mg. of manganese, were titrated.

Copper-Nickel-Chromium Cast Iron 115, Chromium-Vanadium Steel 30d, Chromium-Nickel Steel 101b, and Chromium-Molybdenum Steel 135. Since all these samples contain appreciable amounts of vanadium an oxidizing attack was used in preparing the solutions to make sure that vanadium would finally be present in the +5 state. Five-gram samples were dissolved in 1 to 1 nitric acid with the aid of a minimal amount of hydrochloric acid. The solutions were then boiled down to a small volume with an excess of concentrated nitric acid to reoxidize any vanadium reduced by the hydrochloric acid, and were finally diluted to 250 cc. Aliquots of 50 cc. were titrated.

SUMMARY

A new method is described for the determination of manganese based on the titration of manganous ion with permanganate ion in a pyrophosphate solution of pH 6 to 7, the +2 manganese being oxidized, and the permanganate ion reduced, to a pyrophosphate complex of the +3 state. The titration is performed potentiometrically with a platinum indicator electrode. The potential break at the equivalence point is very large and high precision is attainable. The titration reaction is stoichiometrically exact, and the titrant permanganate solution can be standardized against sodium oxalate or anhydrous potassium ferrocyanide.

Data presented demonstrate that large amounts of chloride, +3 iron, +2 cobalt, copper, nickel, +3 chromium, +6 molybdenum, +6 tungsten, +6 uranium, zinc, aluminum, magnesium, and cadmium do not interfere with the determination of manganese. Nitrate, sulfate, and perchlorate ions are also harmless. When the titration is performed at pH 6 to 7 vanadium interferes if present in large amounts, but amounts of this element up to about one fifth the amount of manganese can be tolerated. The interference of large amounts of vanadium can be circumvented by performing the titration at a lower pH (3 to 3.5).

The method has been applied without any separations to Bureau of Standards samples of manganese ore, ferromanganese, spiegeleisen, manganese bronze, and alloy steels, with results that equal in accuracy those obtainable by the bismuthate method.

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Fractional Separation of Hafnium and Zirconium by Means of Triethylphosphate

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This study is an extension of previous work on separations in which the precipitating ion was not added as such, but formed slowly by hydrolysis in solution. The principles set forth in the original work on the slow formation of hydroxyl ion were confirmed. When the solution is homogeneous with respect to the precipitating ions, the precipitation of a normally gelatinous product may be improved to yield a dense, granular easily filterable precipitate, and a great improvement in the efficiency of separation of difficultly separable elements can be attained. Hafnium was separated from zirconium by fractional precipitation of the ethylphosphates obtained by hydrolysis of triethylphosphate in a boiling solution of 6 *N* sulfuric acid. Data from this separation were used to develop an optimum fractionation procedure, resulting in efficient separation of hafnium from a mixture containing a relatively low percentage of hafnium.

WHEN a precipitation reaction is used for the separation of ions, the formation of a gelatinous precipitate is a serious problem. The slimy nature of the product makes filtration difficult and inefficient washing leaves adsorbed and occluded impurities. If the precipitating ion is not added as such but is formed slowly and homogeneously throughout the body of the reaction mixture, and other conditions are fulfilled, a dense and granular precipitate is formed. This precipitate is easily filtered and washed and carries down few impurities, thus affording a simple means of solving the problem. Willard and his co-workers (*3–6*) successfully carried out the separation of hydroxides and basic salts from many ions by a gradual and uniform increase in pH resulting from the hydrolysis of urea.

This paper extends the work to the formation of ions other than hydroxyl, and the hydrolysis of triethylphosphate is employed successfully in the fractional separation of hafnium and zirconium. Triethylphosphate is completely miscible with sulfuric acid solutions of zirconium and hafnium. When such a mixture is boiled, the ester is hydrolyzed in a stepwise manner and a precipitate of zirconyl or hafnyl ethyl acid phosphate is produced. By using only sufficient ester to precipitate a fraction of the combined hafnium plus zirconium, the hafnium concentrates in the precipitate.

A systematic approach to the separation of zirconium and hafnium by direct fractional precipitation with phosphoric acid was developed by Larsen, Fernelius, and Quill (*1*), in whose paper the references to the literature of the phosphate method are given. The gelatinous nature of the precipitate was overcome by rigid control of the many factors influencing the precipitation reaction. In addition, a method of opening the ore with concentrated sulfuric acid and the solution of the phosphate precipitate were described and are used extensively in the present investigation.

STARTING MATERIAL

The source of the hafnium and zirconium salts was the mineral cyrtolite, an altered zircon containing about 5% hafnia by weight. The ore was crushed, ground to pass 100-mesh, and digested with concentrated sulfuric acid (*1*, *2*). The resulting sulfate solution was then used directly for fractional precipitations or purified by precipitation of the hafnium and zirconium by means of excess triethylphosphate.

METHOD OF ANALYSIS

Two analyses were usually required, one for the combined hafnia and zirconia and the other for the hafnia present in the combined oxides. Whenever possible the determination of combined oxides was run first, the oxides were returned to solution by fusing with potassium bisulfate, and the resulting solution was used for the hafnium determination.

As no simple chemical process is available that will distinguish between hafnium and zirconium, it is necessary to have recourse to some indirect method. One of the most feasible is the conversion of a mixture of two well-defined hafnium and zirconium compounds into two other compounds.

Schumb and Pittman (*2*) published a method of determining hafnium in a mixture of hafnium and zirconium oxychlorides by conversion to the normal selenites followed by ignition to the oxides. Three changes were essential before the method could be applied to the mixtures in this investigation. (1) It was necessary to convert the sulfuric acid solutions of hafnium and zirconium into the corresponding hydrochloric acid solutions. This was accomplished by a double precipitation with ammonia followed by solution in 10 ml. of 1 to 1 hydrochloric acid. (2) It was necessary to destroy the wood sugars resulting from the hydrolysis of the cellulose (filter paper), by boiling the hydrochloric acid solutions with hydrogen peroxide. Following solution in 1 to 1 hydrochloric acid, the solution was boiled with 5 ml. of 30% hydrogen peroxide, and then diluted to 200 ml. for precipitation of the selenites with 50 ml. of 20% selenious acid. (3) It was necessary to remove the silica from the ignited oxides in the combined oxides determination. This was accomplished by evaporating cautiously 10 drops of concentrated sulfuric acid and 0.5 to 1 ml. of hydrofluoric acid from the oxides. The mixed sulfates were then carefully ignited to the oxides, the final ignition being made at 950° C.

NATURE OF PRODUCT SEPARATED BY HYDROLYSIS OF TRIETHYLPHOSPHATE IN PRESENCE OF ZIRCONIUM

If the hydrolysis of triethylphosphate went to completion, zirconyl phosphate, $ZrO[H_2(PO_4)]_2$, would be precipitated. However, the stepwise hydrolysis of the ester results in the precipitation of zirconium ethyl phosphate.

A carefully purified sample was analyzed to obtain the loss on ignition and zirconium and phosphorus contents. On the basis of these results a suggested formula is $ZrO[H(C_2H_5)(PO_4)]_2.2H_2O$. A comparison of the chemical analysis and calculated analysis is made in Table I.

Near the completion of this investigation trimethylphosphate became available and because of its increased rate of hydrolysis over that of triethylphosphate, some experiments were run to compare the two esters. The results indicated that the time required for the same degree of precipitation of zirconium by trimethylphosphate was about one third that required by the triethyl compound. This means a tremendous saving in time, since the usual fractionation procedure to produce relatively pure hafnium compounds with triethylphosphate requires five to six

Table I. Results in Analysis of Zirconium Compound Precipitated by Hydrolysis of Triethylphosphate

	Calculated	Experimental
Mole ratio P/Zr	2.00	2.00
% Zr	23.20	23.12 ± 0.06
% P	15.76	15.76 ± 0.03
% loss on ignition	32.55	32.6 ± 0.1
Molecular weight	393.2	393 ± 1

Table II. Degree of Fractionation in Sulfuric, Nitric, and Hydrochloric Acid Solutions

Type Solution	Mole Fraction $HfO_2 + ZrO_2$ removed	Mole Fraction HfO_2 removed
6 *N* sulfuric acid	0.333	0.623
6 *N* nitric acid	0.333	0.516
6 *N* hydrochloric acid	0.333	0.513

Table III. Effect of Free Acid on Fractionation of Hafnium

Normality of Sulfuric Acid	Initial Weight % HfO_2	Mole Fraction $HfO_2 + ZrO_2$ Removed	Final Weight % HfO_2
6	20.3	0.519	32.9
8	20.3	0.540	29.6
10	20.3	0.525	30.9
12	20.3	0.553	30.9
14	20.3	0.567	30.2

steps at 20 hours per step for the reaction time alone. Also, it is reasonable to expect that the product separated would be analogous to the zirconium ethyl phosphate and therefore would show about the same fractionation characteristics.

EFFECT OF ANION ON FRACTIONATION OF HAFNIUM

The usual explanation of the operation of a fractional precipitation method is based upon the slight difference in the solubility of the products. The ratio of the molecular solubilities of hafnium and zirconium phosphates in 6 *N* hydrochloric acid is only 1 to 4. Zirconium generally preponderates in the usual mixture of zirconium and hafnium, and the repressing effect of the high zirconium concentration should overbalance the slight differences in solubility. The fact that it does not suggests that relative stabilities of anionic complexes may be involved. If this is so, the fractionation should depend upon the anion present in the solution. To check this, the same fraction of the combined oxides was precipitated with triethylphosphate from 6 *N* solutions of sulfuric, nitric, and hydrochloric acids. The results, tabulated in Table II, indicate that the best results are obtained in a sulfuric acid solution.

EFFECT OF FREE ACID CONCENTRATION ON FRACTIONATION OF HAFNIUM

The effect of the free acid concentration was determined by precipitating approximately the same fraction of the combined oxides from solutions of varying sulfuric acid concentration. As shown in Table III, no noticeable improvement in the fractionation takes place as the acid concentration is varied.

6 *N* sulfuric acid was selected as the most desirable reaction medium, primarily for the dense, granular precipitate produced and also because the solutions were the least corrosive.

DEVELOPMENT OF AN OPTIMUM FRACTIONATION PROCEDURE

The development of an optimum fractionation procedure depends upon the application of a suitable criterion for determining the fraction of the total hafnia plus zirconia that should be precipitated at a given step. The highest concentration of hafnia in the product and the greatest recovery of hafnia are the two most important factors to be considered. Unfortunately they are dependent upon one another. If a relatively small amount of the total hafnium plus zirconium is precipitated, the hafnium content of the precipitated phase will be considerably higher than if a larger amount of the combined oxides had been separated. Thus as the yield of hafnia recovered in the precipitated phase is increased, the hafnium concentration is decreased.

At this point a clarification of the term "instantaneous composition" is required. The chemical analysis indicates the average composition over a given interval. If this interval were continually shortened, then at the limit the composition would represent an instantaneous value. Physically it would correspond to the composition of the product separating at a given instant, if all of the previously separated product could be removed.

From the above discussion it is apparent that the instantaneous composition of the separating phase will vary from high values of per cent hafnia to low values as the fractionation progresses. Therefore, the fractionation should be stopped at the point where the instantaneous composition of the separating product is equal to the initial composition of the starting material. To carry the fractionation beyond this point would simply mean that the product was being diluted with a mixture less rich in hafnium than the starting material. Although there is no convenient way to determine experimentally the relation between the instantaneous composition and the fraction of the combined oxides precipitated, it can be deduced by graphical means from other data more easily measured. The first step is a comparison of the fraction of the hafnia precipitated with the fraction of the combined oxides precipitated for initial mixtures containing varying amounts of hafnia, somewhat analogous to the treatment of Larsen *et al.* (*1*).

The experimental work consisted in carrying out a series of single precipitations on starting solutions having the same hafnia content, in which 10 to 70 mole % of the total oxides were precipitated as the ethylphosphates. Sufficient triethylphosphate (375 ml. of ester per mole of oxides) was added to solutions of 6 *N* sulfuric acid, containing 0.1 mole per liter of combined oxides, to precipitate the desired fraction of the oxides as the ethylphosphates. A mole ratio of ester to oxides slightly greater than 2 to 1 was required because of side reactions. The mixtures were boiled for 20 hours, cooled, filtered, and washed with 2% sulfuric acid. Sodium hydroxide–sodium peroxide mixtures were used to decompose the phosphate precipitate (*1*), and make it soluble in sulfuric acid. The resulting solutions were analyzed for both combined oxides and hafnia, from which the desired data were easily calculated. These experiments were repeated for solutions whose combined oxides had different initial hafnia contents. These data are plotted in Figure 1.

For selected values of the abscissa the corresponding ordinates are read from the curves of Figure 1. Then on the basis of 1 mole of combined oxides, the moles of hafnia separated and the

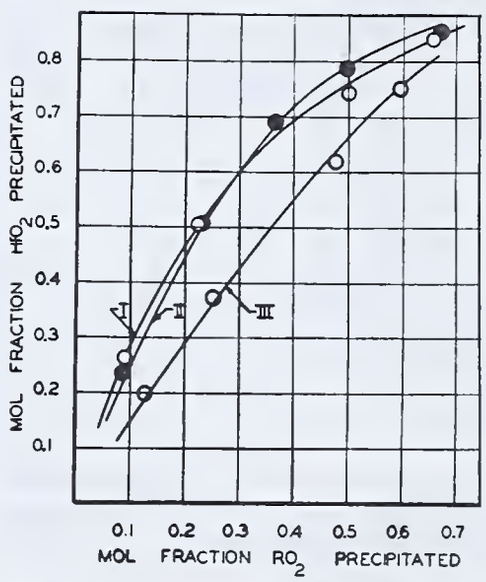

Figure 1. Comparison of Hafnia and Total Oxides Precipitated

Mole Fraction HfO_2 in Starting Material
I. 0.0753
II. 0.179
III. 0.504

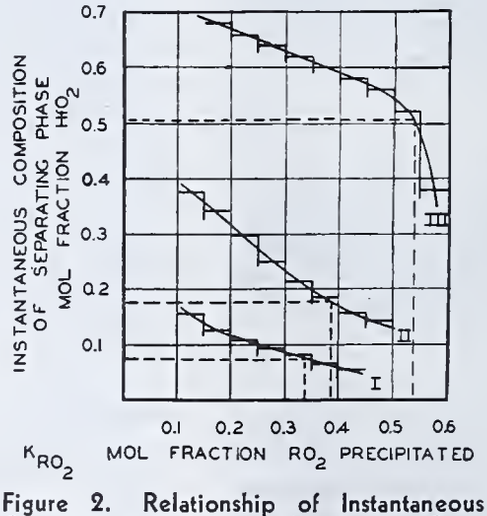

Figure 2. Relationship of Instantaneous Composition and Mole Fraction of Combined Oxides Precipitated

Initial Mole Fraction HfO_2	Optimum Cutoff K_{RO_2}
I. 0.0753	0.34
II. 0.179	0.39
III. 0.504	0.54

Table IV. Fractionation Series Using Optimum Procedure

Fractionation step	Experimental: Weight of RO_2 *Grams*	Experimental: HfO_2 Weight %	Experimental: Solution volume *Ml.*	Experimental: HfO_2 *Mole*	Experimental: Mole fraction HfO_2 precipitated	Experimental: Over-all efficiency HfO_2 precipitated	ZrO_2 *Moles*	RO_2 *Moles*	Composition mole fraction HfO_2	Predicted Composition Mole fraction HfO_2 from Fig. 4	Experimental Mole Fraction RO_2 Precipitated	Desired Mole Fraction RO_2 Precipitated from Fig. 3
1 Initial	215	16.0	4760	0.163			1.464	1.627	0.100			
Final	75.3	30.3		0.1081	0.664	0.664	0.425	0.5331	0.203	0.19	0.328	0.34
2 Initial	74.1	30.3	1525	0.1063			0.418	0.5243	0.203			
Final	29.8	53.6		0.0757	0.712	0.472	0.1120	0.1877	0.403	0.35	0.358	0.38
3 Initial	28.9	53.6	738	0.0735			0.1089	0.1824	0.403			
Final	15.1	75.8		0.0543	0.738	0.348	0.0296	0.0839	0.647	0.57	0.460	0.49
4 Initial	14.0	75.8	435	0.0503			0.0275	0.0778	0.647			
Final	11.65	80.0		0.0442	0.878	0.306	0.0189	0.0631	0.700	0.75	0.812	0.66
5 Initial	10.48	80.0	225	0.0397			0.0170	0.0567	0.700			
Final	7.16	91.1		0.0309	0.779	0.238	0.0052	0.0361	0.853	0.80	0.637	0.71

increments in the moles of hafnia are determined. From these the average compositions of the separating phases corresponding to increments in the precipitation of the combined oxides are calculated and plotted in Figure 2. The instantaneous curve is drawn so that the area under the rectangles equals the area under the curve. The intersection of the instantaneous curve with the ordinate representing the initial composition gives the optimum cutoff in the fractionation. Obviously, if the curve relating the optimum cutoff and the initial composition were plotted, it would have to pass through the points 0,0 and 1,1. These data are made of more general value by putting them on a semilog plot as shown in Figure 3. Thus, for any given initial composition of combined oxides, the optimum extent of precipitation is determined from the curve. Again, considering 1 mole of combined oxides as a basis, and using the curves of Figures 1 and 3, the plot of Figure 4 relating the composition at steps $N + 1$ and N may be obtained. If the experimental data were very accurate and if rigid control could be maintained over the fractionation procedure, this curve would assist greatly in planning the entire fractionation series.

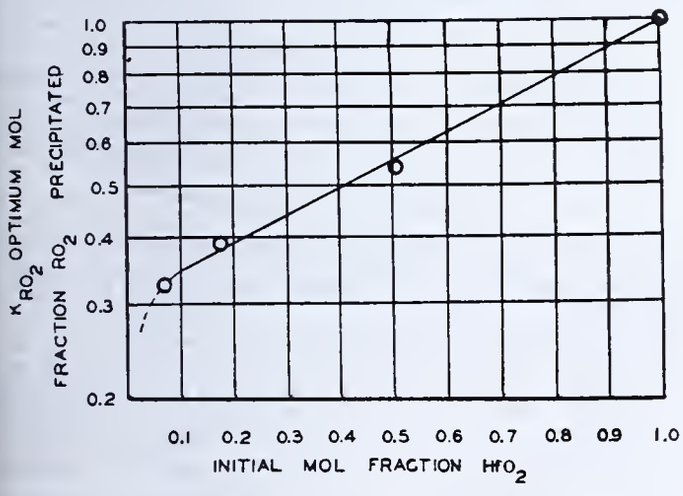

Figure 3. Comparison of Optimum Mole Fraction of Combined Oxides Precipitated and Composition of Starting Material

Using a 45° reference line, a series of steps in the fractionation process could be plotted. Unfortunately, the very small error per step adds to a very considerable cumulative error which greatly lessens its value with the data at present available.

APPLICATION OF OPTIMUM FRACTIONATION PROCEDURE TO FRACTIONATION SERIES

The procedure for using the fractionation curves consists in determining first the initial moles of combined oxides and the hafnia content of these oxides. Then using Figure 3 the desired

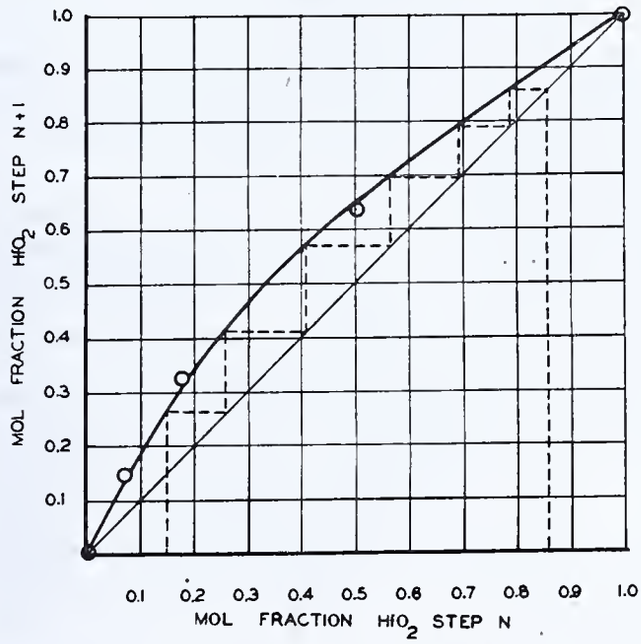

Figure 4. Comparison of Composition at Step $N + 1$ and at Step N

mole fraction of the combined oxides to be precipitated is selected and sufficient triethylphosphate is added to carry out the precipitation. The ethylphosphates are decomposed with a caustic peroxide mixture, and the acid-soluble product is dissolved in 6 N sulfuric acid and analyzed.

From the new analysis the next fractionation step may be planned as before. Figure 4 may be used to predict the composition one or two steps in advance. The data for an entire series are tabulated in Table IV. In this series 215 grams of combined oxides containing 16.0 weight % hafnia were enriched to 7.16 grams of combined oxides containing 91.1 weight % hafnia in five steps. This represents an over-all efficiency in the recovery of hafnia of 23.8%.

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Determination of Tocopherol in Plant Tissue

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Modifications of the Emmerie-Engel and Furter-Meyer methods for the determination of tocopherol are reported, which consist essentially of the removal of substances that interfere with application to plant extracts. In most cases the results obtained by the two procedures agreed within ± 5 to 10%.

DURING the course of investigations on the fat-soluble constituents of vegetable leaf meals, the authors wished to determine the tocopherol content of a number of leaf meals and extracts. A survey of the literature indicated that little work had been done with such materials. The tocopherol content of lettuce leaves has been reported by Karrer and Keller (*8*), and that of alfalfa by Scudi and Buhs (*13*).

The methods used by these workers are lengthy and require specialized apparatus, and attempts to use the latter method with standard tocopherol solutions resulted in poor recoveries. Therefore, the authors attempted to apply the Emmerie-Engel (*3*) and Furter-Meyer (*5*) procedures.

The Furter-Meyer procedure is based on the nitric acid oxidation of tocopherol to the red quinone. The reaction is relatively specific, taking place (*14*) only with compounds that have structures similar to that of tocopherol. The three forms of tocopherol—alpha, beta, and gamma—are not differentiated by this procedure, so that the analytical results show only the total tocopherols present. The method as originally presented by Furter and Meyer was applied only to pure tocopherol solutions and vegetable oils. In leaf extracts carotene, xanthophyll, chlorophyll, tocopherolquinones, and lipoids insoluble in cold ethanol interfered with the determination and had to be removed. In addition Quackenbush *et al.* (*12*) have shown that polyphenols such as pyrogallol interfere with the colorimetric determination. Such compounds, if present, are removed during the purification of the plant extract.

The Emmerie and Engel procedure is indirect. Ferric ion is reduced to the ferrous form by all three tocopherols. The ferrous ion then forms a colored complex with α,α'-dipyridyl. Any compound which can reduce iron will interfere with the method. In leaf extracts carotene, xanthophyll, chlorophyll, and certain lipoids which slowly reduce iron interfere and must be removed.

The modifications of these methods described here consist essentially of the removal of substances which interfere with their application to plant extracts.

EXTRACTION OF TOCOPHEROL

The solutions are kept in the dark except during manipulations and all manipulations are conducted in a room shielded from direct sunlight. All-glass apparatus is used throughout.

DRY SAMPLES. A 25-gram sample of leaf meal, of 30- to 40-mesh, is exhaustively extracted in a Soxhlet with Skellysolve B for 5 to 8 hours, and the extract thus obtained is made to 250 ml. In this manner sufficient sample is obtained for duplicate determinations by both procedures.

FRESH SAMPLES. A 10-gram fresh sample is necessary for the Emmerie-Engel procedure and a 25-gram sample for the Furter-Meyer. The material is extracted in a Waring blender using the foaming mixture of alcohol and Skellysolve B described by Moore and Ely (*9*). This procedure has been successfully used by Devlin and Mattill (*2*) to extract tocopherols from muscle tissue. The alcohol is separated and extracted with Skellysolve B as described by Wall and Kelley (*15*). The Skellysolve solution is then dried with anhydrous sodium sulfate and evaporated under vacuum and nitrogen to approximately 25 ml.

FURTER-MEYER PROCEDURE

The sample taken should contain a minimum of 0.3 mg. of tocopherol and preferably about 1.0 mg. An extract equivalent to 2.5 to 5.0 grams of dry leaf meal usually contains the necessary quantity of tocopherol. The sample dissolved in about 25 ml. of Skellysolve B is passed through a 7 × 2 cm. adsorption column consisting of three parts of Hi-Flow Supercel (Johns-Manville Corporation) and one part of activated magnesia No. 2641 (Westvaco Chlorine Products Company, Newark, Calif.) The preparation of the column and the adsorption technique are identical with those described by Wall and Kelley (*15*) for the determination of carotene. After adsorbing the extract, the adsorption column is washed with about 80 ml. of 5% acetone in Skellysolve B and finally with 20 ml. of 10% acetone in Skellysolve B. The carotene and tocopherol are eluted, chlorophyll and xanthophyll being retained on the column. The eluate is caught in a 250-ml. flat-bottomed boiling flask, ₹24/40.

The solution is evaporated in a water bath at 40° to 50° C. under vacuum and nitrogen to approximately 25 ml. To the concentrated solution are added 2.0 ml. of 85% (by volume) sulfuric acid. The flask is stoppered and vigorously shaken on a shaking machine for 3 minutes. The acid is colored deep blue by the decomposed carotene. Occasionally, if all the pigment is not removed, 2.0 ml. more of acid must be added. The stopper is removed and rinsed with Skellysolve B, and the contents of the flask are rinsed into a 250-ml. separatory funnel with Skellysolve B. The acid layer which separates is withdrawn. The Skellysolve solution is washed once with aqueous 5% sodium sulfate solution, then once with aqueous 1% potassium hydroxide, and finally with more sodium sulfate solution until the aqueous washings are practically neutral.

The solution, which should be colorless or only slightly yellow, is dried with anhydrous sodium sulfate, decanted from the sodium sulfate, and evaporated almost to dryness. The residue is transferred with 3 or 4 washes of Skellysolve B to a 50-ml. Erlenmeyer flask, ₹19/22, and the solution is again evaporated just to dryness. To the flask are added 5.0 ml. of absolute ethanol and then, while the flask is swirled, 1.0 ml. of concentrated nitric acid. The flask, attached to a small Liebig condenser by means of lightly greased interchangeable joints, is then set in a steam bath. The alcoholic solution is refluxed for exactly 3 minutes from the time the alcohol begins to boil. The flask is taken from the steam bath, and cooled with cold water while still connected to the condenser. The flask is then removed, stoppered, and kept in the dark while the color develops. This takes a minimum of 15 minutes; longer periods do not affect the determination.

The nitric acid oxidation of tocopherol results in the formation of a red tocopherolquinone. Prior to making the colorimetric measurements, lipoidal constituents of plant extracts insoluble in cold alcohol must be removed. The alcoholic solution is filtered on a small, dry, sintered, medium porosity Hirsch funnel. A micro colorimeter tube is placed inside a suction flask with the tip of the funnel just inside the tube. The solution is rapidly filtered with minimum suction (pressure filtration may also be used for this operation), the funnel being kept covered with a watch glass to minimize evaporation. The colorimeter tube is then removed and stoppered.

The colorimetric measurement was made with a Fisher Electrophotometer, at a wave length of 480 mμ. To secure this wave length a Corning filter combination consisting of one No. 3389 and two No. 4303 filters is used. A blank composed of 5.0 ml. of absolute ethanol and 1.0 ml. of concentrated nitric acid is the reference solution. Because the blank slowly darkens, it should not stand more than 0.5 hour.

A standard calibration curve, shown in Figure 1, A, was made with solutions of Merck synthetic α-tocopherol in Skellysolve B. The standard solutions were evaporated, oxidized with nitric acid, and filtered as described previously.

EMMERIE-ENGEL PROCEDURE

The best concentration range for this procedure is 0.05 to 0.20 mg. It is more convenient to purify a larger sample containing 0.2 to 1.0 mg. and then take a suitable aliquot for the final determination.

A Supercel–activated magnesia column approximately 4.0 × 2.0 cm. is prepared as described. The sample is adsorbed, eluted, and concentrated as in the Furter-Meyer procedure. After the sample is shaken for 3 minutes with 1 to 2 ml. of 85% sulfuric

Table I. $E^{1\%}_{1\ cm.}$ and 480 and 520 mμ Values for Synthetic α- and Natural α- and γ-Tocopherols

Compound	Furter-Meyer Method			Emmerie-Engel Method		
	Mg.	$E^{1\%}_{1\ cm.}$, 480 mμ		Mg.	$E^{1\%}_{1\ cm.}$, 420 mμ	
Synthetic α-tocopherol	1.0	15.5	15.5	0.1	238	238
	2.0	15.5		0.2	238	
Natural α-tocopherol	1.0	16.5	16.4	0.1	249	242
	2.0	16.3		0.2	234	
Natural γ-tocopherol	1.0	13.5	13.9	0.1	174	152
	2.0	14.2		0.2	130	

cid, it is transferred to a separatory funnel and treated as described above. After the last sodium sulfate wash, as much aqueous solution as possible is removed and, without drying, the purified Skellysolve solution is run into a 100-ml. volumetric flask and made to volume.

A 25-ml. aliquot is evaporated almost to dryness on a warm water bath under vacuum and nitrogen. The residue is dissolved in 10 ml. of chloroform and transferred with three chloroform washes totaling 20 to 30 ml. to a 125-ml. separatory funnel. To the chloroform solution are added 1.0 ml. of a 0.5% α,α′-dipyridyl solution in absolute ethanol and 1.0 ml. of a 0.4% ferric chloride solution in absolute ethanol. The reagents are run down the sides of the funnel to minimize mixing. The funnel is then shaken vigorously, approximately 10 ml. distilled water are added at once, and the funnel is again shaken. The stopper and sides of the funnel are then rinsed with a few milliliters of absolute alcohol. Two layers form. The lower chloroform layer is discarded; the upper aqueous alcohol layer, which contains the red ferrous dipyridyl color complex, is run into a 25-ml. volumetric flask and made to volume with absolute ethanol.

The time for color development is 10 minutes, measured from the time the reagents are first mixed with the tocopherol solution. The colorimetric measurement was made with a Fisher Electrophotometer at 520 mμ, obtained by a combination of three Corning filters, one No. 4380, one No. 4303, and one No. 3384. A blank containing 10 ml. of distilled water diluted to 25 ml. with absolute ethanol is used.

A calibration curve was made with Merck synthetic α-tocopherol in Skellysolve B solution. Suitable aliquots were evaporated, and the color was developed as described above. A ferric chloride–α,α′-dipyridyl reagent blank obtained in the same manner as the standards was determined, and the value thus obtained was deducted from the standard readings. Calibration curves obtained before and after deducting the reagent blank are shown in Figure 1, B. Either calibration curve may be used.

DISCUSSION AND RESULTS

Effect of Tocopherol Mixtures. The procedures presented were based on colorimetric standardization with Merck synthetic α-tocopherol. Baxter *et al.* (*1*) and Hove and Hove (*7*) have shown that α-tocopherol is oxidized more rapidly by ferric chloride than are the beta and gamma compounds. On the other hand, when the tocopherols are oxidized to the red *o*-quinone with silver nitrate, this order is reversed (*1*)

In view of the fact that γ-tocopherol might be present in plant tissue and that the synthetic and natural tocopherols might behave differently, the $E^{1\%}_{1\ cm.}$ and 480 and 520 mμ values for synthetic α- and natural α- and γ-tocopherols were determined by modifications of the Furter-Meyer and Emmerie-Engel procedures (Table I).

The $E^{1\%}_{1\ cm.}$ values obtained by both methods for synthetic and natural α-tocopherol agree fairly well, the result for natural tocopherol being about 5% high in both cases. As compared with synthetic α-tocopherol, the natural gamma compound gave values that averaged 10% low by the Furter-Meyer and 35% low by the Emmerie-Engel method. Therefore if γ-tocopherol were present in appreciable quantity in leaf material, the error in colorimetry alone would be serious, especially in the Emmerie-Engel determination. As is shown below, in most cases results by the two procedures were in close agreement, indicating that there was little, if any, γ-tocopherol in the products studied.

In this connection, recent papers by Hove and Hove (*7*) and Fisher (*4*) describe the determination of α-tocopherol in the presence of the beta and gamma compounds. It is possible that these procedures in conjunction with the methods described here may be used to determine the individual tocopherol compounds present in plant tissue.

Removal of Pigments. Passage of the extract through activated magnesia not only effectively removes the chlorophyll and xanthophyll but allows quantitative elution of the tocopherol, together with the carotene. Table II shows the results of adsorption experiments with synthetic α- and natural α- and γ-tocopherols. After elution with the acetone–Skellysolve B solutions described previously, the colorimetric determination was made by the Furter-Meyer method, individual calibration curves prepared from each tocopherol compound being used. The results indicate that all the tocopherols can be eluted quantitatively from the magnesia adsorbent.

Table II. Effect of Adsorption and Acid Treatment on Recovery of Synthetic α- and Natural α- and γ-Tocopherols

Compound	Adsorption			Sulfuric Acid Treatment		
	Taken	Found	Recovery	Taken	Found	Recovery
	Mg.	Mg.	%	Mg.	Mg.	%
Synthetic α-tocopherol	2.00	2.00	100	2.0	1.90	95.0
Natural α-tocopherol	2.00	2.03	101.0	2.0	1.95	97.5
Natural γ-tocopherol	2.00	2.05	102.5	2.0	1.90	95.0

Attempts at separating carotene from tocopherol by adsorption on Florisil from benzene solution were unsuccessful. The quantities of carotene and other lipoids are much larger in leaf extracts than in oil and animal tissue extracts, which have been successfully treated with Florisil.

Carotene is removed in both methods by 85% (by volume) sulfuric acid. Parker and McFarlane (*11*) used 85% by weight sulfuric acid to remove the small quantities of carotenoids present in oils. In order to remove the carotene quantitatively, it is necessary to work with a concentrated solution, which must be vigorously shaken with the acid. Under these circumstances the sulfuric acid rapidly decomposes the carotene, and the resultant petroleum ether solution is colorless. Tocopherol losses in this case are low. If the solution is not concentrated, prolonged shaking with the acid is required. Often much larger quantities of acid are necessary. The tocopherol losses in this case amounted to 25 to 50% with the Emmerie-Engel procedure, probably due to oxidation; they were somewhat lower with the Furter-Meyer method. Table II shows the effect of the 85% sulfuric acid treatment on the recovery of snythetic α- and natural α- and γ-tocopherols. The acid treatment was identical with the procedure described above. The colorimetric determination was made by the Furter-Meyer method, individual calibration curves being used. The results show that the acid treatment caused a maximum loss of 5%.

According to Scudi and Buhs (*13*), treatment with 85% sulfuric acid also removes tocopherolquinones, which would otherwise be included with tocopherol values by the Furter-Meyer method. The authors' observations with pure α-tocopherolquinone confirm this. Water-soluble polyphenols, if present, are removed by the water and alkali shakings subsequent to the treatment of the extract with 85% sulfuric acid.

Effect of Unknown Lipoidal Interferences. After the acid treatment, the only subsequent interference observed in the Furter-Meyer procedure came from lipoidal compounds insoluble in cold ethanol. When nitric acid is added to the ethanol containing the tocopherols, and the solution boiled, a clear red product is obtained. On cooling, however, a considerable quantity of material is usually precipitated. The solution thus

becomes turbid and colorimetric readings taken with such a solution would be erroneous. These lipoids are easily removed by filtration, leaving a clear solution. In the Emmerie-Engel procedure, several interfering substances of unknown nature are left after adsorption and acid and alkali treatments. One source of interference is a lipoid which slowly reduces iron.

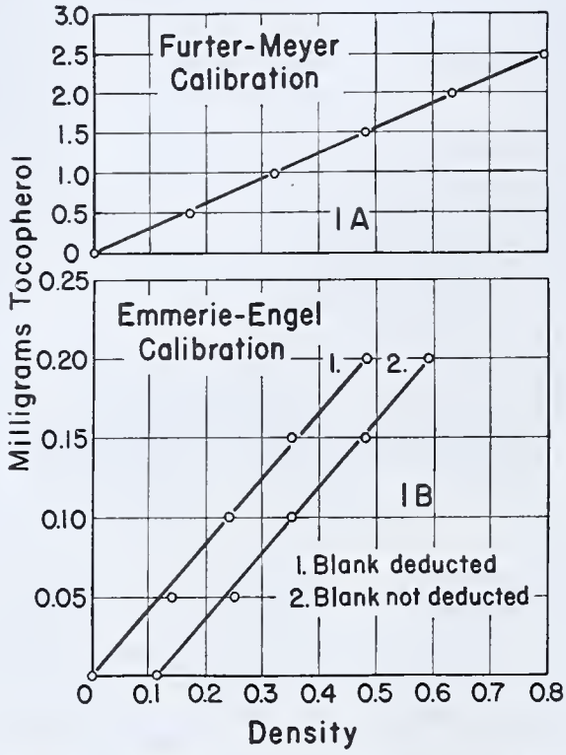

Figure 1. Calibration Curve for Determination of Tocopherol

Purified plant extracts transferred to alcohol and then reacted with the Emmerie-Engel reagents reduced the iron at a slow rate for periods up to 2 or 3 hours. Under the same circumstances pure tocopherol gave a constant reading in 10 minutes. The slow reduction of iron was not linear, for extracts containing twice the quantity of sample gave results 150 to 200% higher than the calculated results. The same effect was noted when pure tocopherol was added to a plant extract, the tocopherol recovery being 150 to 200% higher than the calculated value. The interfering lipoids may be plant sterols, for Devlin and Mattill (*2*) working with muscle extracts noted the same type of interference with the Emmerie-Engel procedure and traced the effect to cholesterol. The authors completely eliminated this source of interference by immediately transferring the colored dipyridyl complex to an aqueous medium, thus taking the lipoidal reducing substances out of contact with the iron. Pure α-tocopherol reduced the iron immediately. Experiments with pure tocopherol solutions treated in this manner gave practically quantitative recovery.

Still another type of interference was noted with certain plant extracts, particularly beet and rhubarb extracts. Recovery of tocopherol added to these extracts and carried through the whole procedure was consistently low. When tocopherol was added directly to the extract after purification of the sample, the recovery was still low. Working on the assumption that some constituent of the purified extract was forming a complex with the ferric ion, the concentration of the iron reagent was doubled (from 0.2 to 0.4%), the quantity of sample used for the final tocopherol determination being kept at a minimum. This technique resulted in greatly improved tocopherol recoveries and was adopted for all plant extracts.

Since the high blank shown in Figure 1, B, may have been due to the exposure to suffused light during the manipulation or to the higher concentration of ferric chloride, these two factors were examined. The densities of blank solutions prepared in the conventional manner were compared with those of solutions so handled that there was no exposure to light. The results in both cases were identical.

To determine the effect of ferric chloride concentration, a series of pure synthetic α-tocopherol standards was run by the conventional procedure, using in one case 1 ml. of 0.2% and in the other 1 ml. of 0.4% ferric chloride solution. The results are shown in Table III.

It is apparent that the increased blank is due to the higher ferric chloride concentration. If the blanks are deducted, the results with either concentration are in good agreement. Although a large blank is generally undesirable, it is necessary to use the high ferric chloride concentration for reasons explained above, and apparently there is no loss of accuracy.

Recovery of Tocopherol. Recovery of synthetic α-tocopherol added to plant extracts and carried through the entire procedures is shown in Table IV. In most cases recovery of tocopherol added to a wide variety of vegetable leaf extracts was satisfactory, averaging about 95% with both methods.

Recovery of tocopherol added to a plant extract is probably more a measure of the precision of the method than of its intrinsic accuracy. The fact that tocopherol is determined rather accurately by the two methods is shown by the fairly good agreement of values obtained by them on the same plant materials (Table V). With a few exceptions, the two methods give results checking within ±5 to 10%. In those few cases in which there is a large discrepancy, the values obtained by the Furter-Meyer method are probably more reliable, since this determination is based on a reaction relatively specific for tocopherol.

Only a few analyses reported in the literature compare the results obtained by the Furter-Meyer and the Emmerie-Engel procedures on biological material. Morton (*10*) quotes an analysis of an oil in which the Furter-Meyer result was 400% higher

Table III. Effect of Iron Concentration on Reagent Blanks Found with Emmerie-Engel Method

	0.2% Ferric Chloride		0.4% Ferric Chloride	
Tocopherol	Density	Density (blank deducted)	Density	Density (blank deducted)
0	0.08	0.00	0.15	0.00
50	0.21	0.13	0.27	0.12
100	0.32	0.24	0.38	0.23
200	0.55	0.47	0.61	0.46

Table IV. Recovery of α-Tocopherol Added to Plant Extracts

Dry Leaf Sample	Tocopherol in Sample	Tocopherol in Sample Plus 1.0 Mg. of Added Tocopherol: Calculated value	Observed value	Total Tocopherol Recovered
	Mg.	*Mg.*	*Mg.*	%
	Furter-Meyer Method			
Alfalfa	0.47	1.47	1.38	93.8
Beet	1.19	2.19	2.04	93.0
Broccoli	1.25	2.25	2.22	98.6
Kale	0.91	1.91	1.80	94.2
Lima bean	1.79	2.79	2.68	96.0
Rhubarb	2.74	3.74	3.64	97.3
Spinach	1.29	2.29	2.24	97.8
Turnip	0.85	1.85	1.78	96.2
				Av. 95.8
	Emmerie-Engel Method			
		Plus 62.5 γ Added Tocopherol		
	γ	γ	γ	
Alfalfa	65.0	127.5	133.0	104.3
Beet	89.0	151.5	147.0	97.0
Broccoli	100.0	162.5	149.0	91.7
Kale	97.0	159.5	144.0	90.0
Lima bean	175.0	237.5	221.0	93.0
Rhubarb	150.0	212.5	213.0	100.2
Spinach	100.0	162.5	155.0	95.3
Turnip	88.0	150.5	152.0	100.9
				Av. 96.5

Table V. Tocopherol Content of Vegetable Tissues

Sample	Furter-Meyer	Emmerie-Engel
	Mg. per gram	
Alfalfa leaf meal	0.23	0.26
Beet leaf meal	0.44	0.71
Broccoli leaf meal	0.39	0.42
Kale leaf meal	0.36	0.39
Lima bean leaf meal	0.72	0.70
Rhubarb leaf meal	1.24	1.20
Spinach leaf meal	0.42	0.40
Turnip leaf meal	0.34	0.30
Mash, 5% alfalfa	...	0.02
Mash, 2.5% broccoli	...	0.02
Scratch corn	...	0.00
Fresh carrot root	0.14[a]	0.13[a]
Fresh carrot top	0.79[a]	0.56[a]
Frozen spinach	0.47[a]	0.43[a]

[a] Calculated on moisture-free basis.

han the Emmerie-Engel value. Hickman *et al.* (*6*) report an nalysis of a mixture of foods in which the Furter-Meyer value as 1400% higher than the Emmerie-Engel result. These extreme discrepancies are undoubtedly due to insufficient purification of the extracts prior to color measurement.

The Emmerie-Engel procedure is much more sensitive than the 'urter-Meyer, and hence is more convenient for low potency amples.

SUMMARY

The Furter-Meyer and Emmerie-Engel methods for determination of tocopherol have been applied to plant extracts. In both ases, dry plant materials are extracted with Skellysolve B, and resh materials with an ethanol–petroleum ether solution, the lcohol then being removed. It is necessary to purify the sample before the final estimation can be made. Chlorophyll and anthophyll are separated from tocopherols by adsorption on a Supercel–activated magnesia column. Carotene and tocopherolquinones are then destroyed by treatment with 85% sulfuric acid.

In the Furter-Meyer procedure the tocopherols are finally ransferred to ethanol solution, oxidized with nitric acid, and, fter removal of ethanol-insoluble lipoids, determined with a photoelectric colorimeter at 480 mμ.

In the Emmerie-Engel procedure the tocopherols are finally dissolved in chloroform, and reacted with the ferric chloride–α,α′-dipyridyl reagent. The pink ferrous dipyridyl complex is immediately transferred to aqueous solution to prevent further reduction of the iron by a slowly reducing fat-soluble compound. The colorimetric determination is carried out at 520 mμ in a photoelectric colorimeter. $E_{1\text{ cm.}}^{1\%}$ and 480 and 520 mμ values obtained by the two methods with synthetic and natural α-tocopherols agreed within 5%. The values for natural γ-tocopherol were 10% lower by the Furter-Meyer method and 35% lower by the Emmerie-Engel method. All these compounds can be quantitatively eluted from a magnesia-Supercel adsorbent and show a maximum loss of 5% when shaken with 85% sulfuric acid.

Tests in which pure synthetic α-tocopherol was added to the extracts and carried through the entire procedure showed an average recovery of 95% with both methods. In most cases the results obtained by the two procedures agreed within ±5 to 10%.

ACKNOWLEDGMENT

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Method of Evaluating Metal Cleaners

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A quantitative method for performance evaluation of alkaline metal cleaners is described and discussed. Reproducibility is rather good. The method involves coating of metal panels with various oils by a specific dipping and drainage technique, followed by a carefully controlled cleaning and rinsing procedure. The panels are covered with a fine spray of water, which condenses as droplets on the oil-covered areas, providing a pattern that remains constant for a sufficient time for a sketch to be drawn on paper divided into 100 squares. The average value for cleaned area of 5 panels is the cleaning efficiency index. Conditions influencing results and variations in the procedure are discussed.

THE importance of having an adequate method for evaluating alkaline cleaners for the removal of contaminants from metal surfaces is generally agreed upon. Morgan and Lankler made an important contribution in this direction (*3*) in 1942 in devising a semiquantitative method that involved photographing fluorescent oil residues under ultraviolet light after a standard cleaning procedure. It was applied specifically to the removal of mineral oil by alkaline salts containing a surface-active agent of the sodium keryl benzene sulfonate type (*2*). This procedure is rather unwieldy, particularly for the control and procurement of alkaline cleaners.

The "water-break" method has been used for a long time as a criterion for evaluating metal surface cleanliness. This test is based on the ability of metal surfaces to sustain an unbroken film of water when "chemically" clean. It has not been found adequate, since the water-break pattern was observed to be dependent on the thickness of the water film. Smaller and smaller areas sustaining a complete water film were obtained as the water drained from the panel. As these areas began to reach a more or less steady state, evaporation of the water became a factor in obscuring the results. As a result of these factors, evaluations performed with the water-break test, as normally used, did not provide an adequate estimate of the efficiency of metal cleaners.

For these reasons, a method has been devised which is fairly simple and has been found capable of yielding results of good reproducibility.

PROCEDURE

Some of the modifications desirable for application to specific problems are obvious. In general, the method used at the arsenal

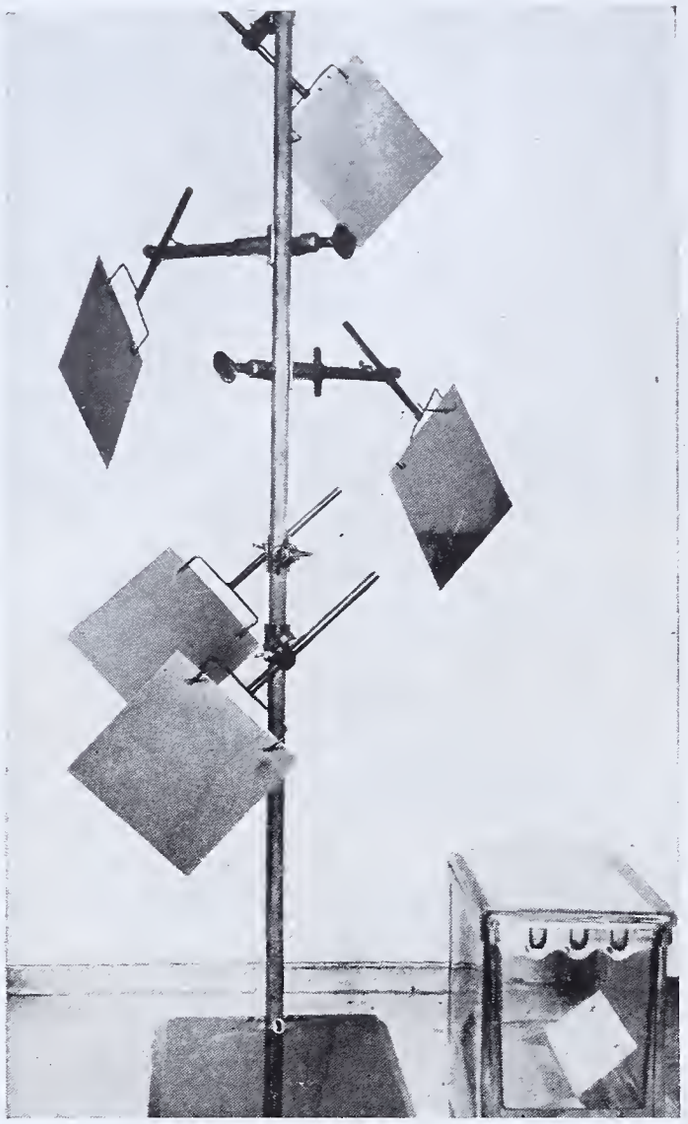

Figure 1. Coating Technique

involves coating of panels by a controlled dipping and drainage technique, followed by carefully controlled cleaning and rinsing operations. The panels are then subjected to a fine spray of water, which has the effect of sharply delineating the oil-covered regions. Quantitative estimation is made by sketching this pattern on paper divided into squares and counting the number of squares that are free of oil.

Preparation of Panels. The panels are of light-gage metal, 10 cm. (4 inches) square, of cold-rolled SAE 1010 steel. Cold-rolled brass or aluminum may also be used. Two holes are drilled in each panel to enable the insertion of a 2-pronged panel holder (Figure 1) which maintains the panel in position without the use of nuts and bolts or similar arrangements. This was found necessary because other holders trapped some of the oil, which afterwards spread and gave erroneous values. The steel panels are degreased before use with an alkaline silicate plus a synthetic detergent and rinsed with water until a complete water film is sustained. They are then pickled at room temperature, for 1 minute, in 6 N hydrochloric acid containing a small amount of wetting agent, rinsed in running cold water for a short time, given two successive rinses in hot alcohol containing 1% ammonia (70° to 80° C.), air-dried, and stored in an evacuated desiccator until used.

Coating of Panels. The panel is immersed in the oil to about half its height. The container is tilted so that the entire panel is covered with oil and then returned to an upright position such that the excess of oil drains from around the holes in the panel. The panel holder prongs are inserted and the panel is hung in a rack at a 45° angle (see Figure 1).

The panels are allowed to drain at 25° ± 2° C. for 1 hour. The globule of oil that remains on the bottom corner is removed. The panels should be shielded from drafts during the drainage period.

It is desirable to determine the weight of oil on individual panels at frequent intervals to ensure control over these conditions.

Two oils are used for coating panels. In one case, the coating is obtained from a hydraulic mineral oil of high viscosity index, with a viscosity at 100° F. of 470 seconds Saybolt Universal viscosity. In the other case, the coating is obtained from a solution of a sulfurized lard oil, containing approximately 12% sulfur, dissolved in toluene to the extent of 1 part of sulfurized oil to 9 parts of toluene. In the work performed with this procedure, the average weight of oil on each panel after coating with mineral oil was 0.283 gram and after coating with sulfurized fatty oil was 0.038 gram. Maximum variations were ±4% for the mineral oil and ±6% for the sulfurized fatty oil, with the great majority of the weights varying from the average by no more than 2%.

Alkaline Salt Solutions. The alkaline salts and surface-active agents are dissolved in water to make 2 liters of solution of appropriate concentration. Solutions of 1.5 or 3.0% of alkaline cleaner are usually employed. The beaker containing the solution is heated and then placed in a constant-temperature bath in which the temperature of the solution is maintained at 60° ± 1° C.

Cleaning and Rinsing Operations. The panel holder is inserted through a cover which tends to reduce evaporation, and connected to a small motor (Figure 2). The panel is positioned in the cleaner so that at least 2.5 cm. (1 inch) of the solution are above the top of the panel, to reduce the effect of temperature changes near the surface. The motor is operated at 10 r.p.m. and the cleaning process is carried on for 5 minutes. The panel is then withdrawn and placed in a tank of running water maintained at 50° C. It is withdrawn from the water once each minute during a rinsing period of 5 minutes. The panel is then rinsed in water at room temperature for 1 minute and allowed to drain for 1 minute.

Quantitative Evaluation. A fine spray of water is directed at the panel from a distance of approximately 60 cm. (2 feet) by means of an atomizer connected to a compressed air line. (The head of a De Vilbis bulb atomizer was attached to a compressed air line equipped with a reducing valve. A small spray gun, such as is used for spraying paint, was also employed. The air is filtered through glass wool.) The oil-covered regions are delineated by the condensation upon them of fine droplets of water, resulting in a pattern which remains constant for at least 20 minutes (Figure 3). These are then sketched on paper ruled into 100 squares (Figure 4). The number of squares covered with water (no droplets condensed) is counted for each side of the panel. The results obtained with 5 panels, resulting in 10 observations, are averaged and this is called the cleaning efficiency index. The standard deviation is determined by the formula:

$$\text{Standard deviation} = \sqrt{\frac{\Sigma d^2}{n-1}}$$

d = deviation of individual values from the mean
n = number of observations

Individual values deviating from the mean by more than three times the standard deviation are discarded. The entire determination is discarded when the standard deviation is greater than 9. A large value for the standard deviation was found to be due to

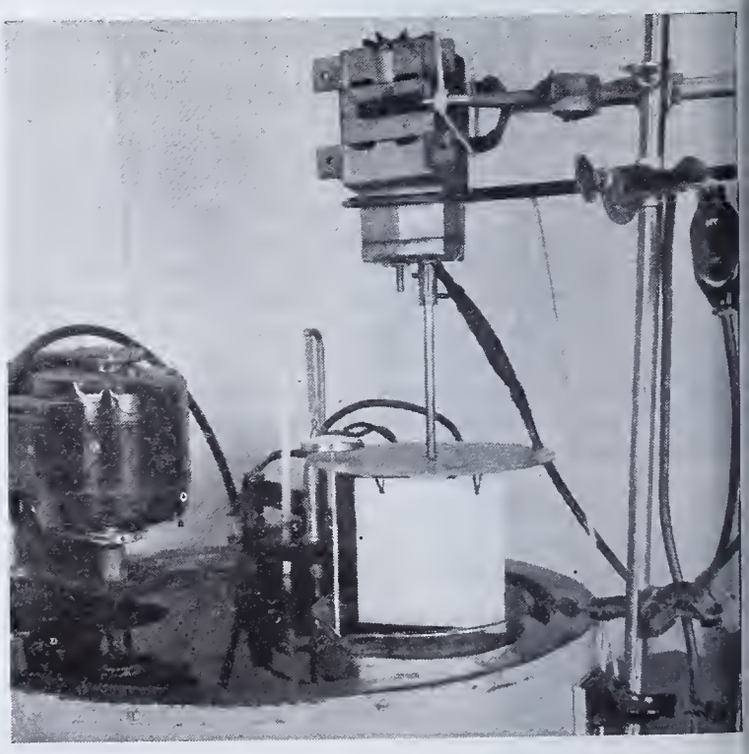

Figure 2. Assembly before Test

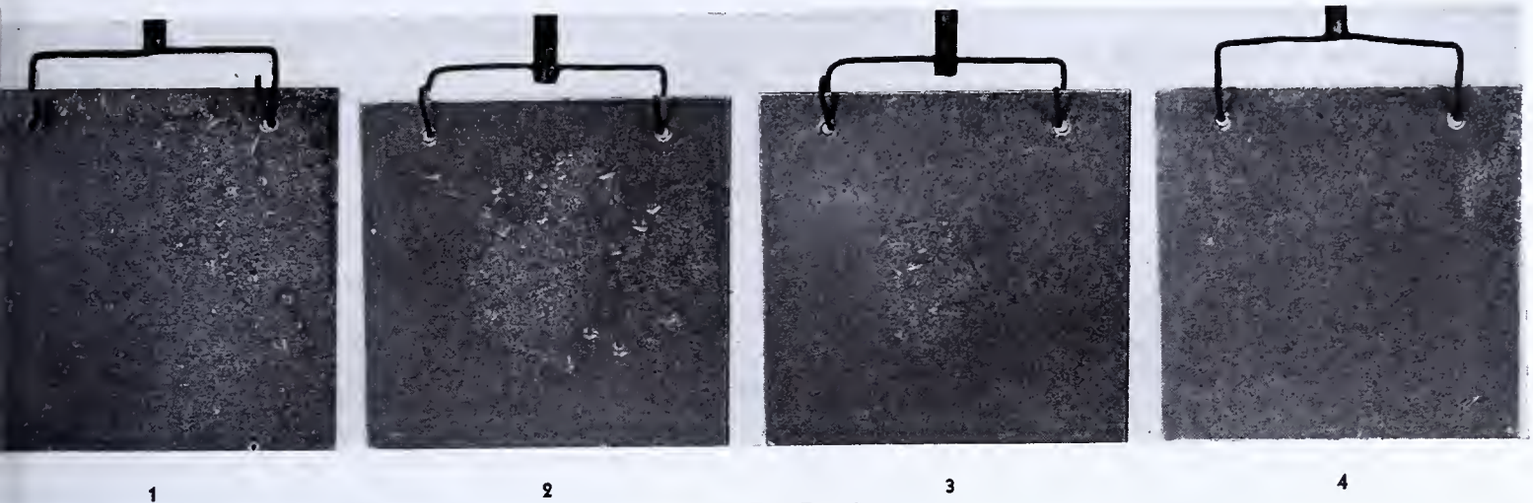

Figure 3. Test Panels

1. Poor cleaning (cleaning index 6) 2. Fair cleaning (cleaning index 73) 3. Fair to good cleaning (cleaning index 88) 4. Good cleaning (cleaning index 94)

Table I. Effect of Surface Smoothness of Aluminum Panels

Oil	Cleaning Index: Aluminum (mirror finish)	Cleaning Index: Aluminum (roughened)
Light mineral oil	99	89
Heavy mineral oil in toluene (1 to 3)	97	11
Lard oil (Prime No. 1)	94	17
Sulfurized mineral oil	95	23
Sulfurized fatty-mineral oil	88	2
Sulfurized fatty base in mineral oil	84	11

ariations in the temperature of the room during the drainage of il from the panels. The frequency of this occurrence, however, is ot sufficient to interfere with the usefulness of the method.

DISCUSSION

Selection of Panels. The surface condition of metal panels as a considerable bearing on cleaning efficiency. In utilizing this est to solve specific problems, therefore, panels should be selected ith surfaces similar to those involved in the problem. So far s the test itself is concerned, the conditions listed above are out f the critical range for surface variations.

Coating of Panels. It was observed that the ease of removal f oil from the panels was dependent to some extent upon the oil lm that was used. Thus, specific differences were observed beween sulfurized fatty base oils and mineral or lard oils under ertain conditions. Another very important factor was the viscosity of the oil used. It may be seen from Table II that the ease f oil removal decreased as the viscosity of the oil and the weight f oil on the panel increased. The distribution of oil on each of he panels is not uniform, since during the drainage period a reater amount of oil is concentrated on the lower portion of the anel. In a great many tests, however, it was observed that results were not consistently poorer for he lower half of the panel than for the top half, ven though approximately two thirds of the total il was on the bottom half of the panel.

One of the factors that controlled the reproducibility of results to a great extent, in utilizing the bove coating procedure, was the temperature at hich the excess oil was drained from the panel. n order to obviate difficulties due to this, it was ound necessary to maintain the drainage temperature within ±2° C. at 25° C. during the 1-hour eriod. Examination of the data in Table III indicates that in this range the temperature effect is less han that due to random variables. Lower temperatures resulted in erratic results; higher temperatures n better results which were reproducible. After considerable experience with a particular set of conditions, it should be possible to apply a correction factor for various room temperatures during the hour-long drainage period.

Cleaning and Rinsing Operations. A temperature of 60° C. (140° F.) was utilized in the procedure. This temperature is somewhat lower than that recorded for most industrial cleaning operations, although it is not uncommon for the temperature of production cleaning tanks to drop to this level. Cleaning efficiency improved rapidly as the temperature of cleaning was increased above 60° C. This tended to obscure differences among cleaners and the various factors affecting cleaning.

The effect of agitation, up to 36 revolutions per minute, was found to be fairly small. Generally, the effect of concentration of alkali was considerable up to a minimal value, beyond which large increases in concentration caused relatively small improvements in cleaning efficiency. The concentration of 3% alkaline salts in the above procedure was found to be above this minimum concentration for most conditions. Lower concentrations of alkaline salts tended to accentuate differences among surface-active agents.

Table II. Effect of Viscosity of Mineral Oil on Cleaning Efficiency

Viscosity at 100° F.	Cleaning Index	Weight of Oil on Each Panel	No. of Determinations
Sec. S.U.V.		*Gram*	
970	11	0.454	2
651	42	0.378	7
456	55	0.336	6
283	95	0.265	6

Cleaner, 3% sodium orthosilicate plus 0.15% sodium keryl benzene sulfonate (40%).
Conditions, 60° C., 10 r.p.m., 5 minutes.

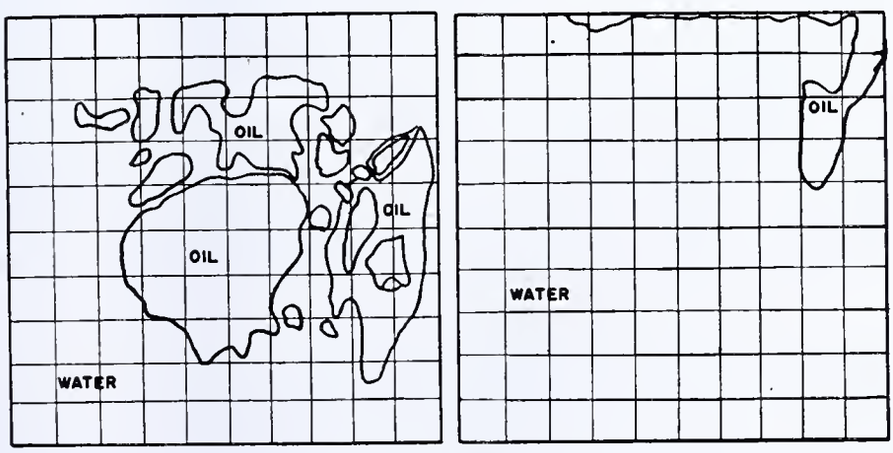

Figure 4. Cleaning Results

Left. Cleaning index 73 (fair). *Right.* Cleaning index 94 (good)

Table III. Typical Reproducibility Data

	Temp., ° C.	Medium Mineral Oil[a] Cleaning efficiency index	Medium Mineral Oil[a] Standard deviation	Sulfurized Fatty Oil Base[b] Cleaning efficiency index	Sulfurized Fatty Oil Base[b] Standard deviation
1	25	56	6	80	9
2	25	53	5	86	8
3	23	55	5	85	5
4	24	53	3	81	6
5	24	55	5	83	6
6	26	56	8	85	6

[a] Mineral oil of high viscosity index. Viscosity 470 sec. per 100° F. S.U.V. Av. weight 0.283 gram.
[b] Sulfurized fatty oil in toluene (1 to 9). Av. weight 0.038 gram. Temperature between 23° and 27° C.
Cleaner, 3% sodium orthosilicate plus 0.15% sodium keryl benzene sulfonate (40%). Conditions, 5 minutes, 10 r.p.m., 60° C.

Variations in the rinse-water temperature were found to be not very important. In the procedure a fairly long rinsing time is provided at a fairly high temperature. Under these conditions it was found unnecessary to dip the panels in dilute acid, prior to the evaluation, to avoid "false water film continuity" (*1*) due to the presence of some of the surface-active agent. The main reason for eliminating the acid rinse was that rusting of the panel developed after this rinse and tended to obscure the patterns of oil-covered areas that were being sketched.

Evaluation and Reproducibility. While the spraying of the panel with water is not extremely critical, care should be exercised to prevent its becoming drenched with a large excess of water. Two or three fairly slow passes with a fine spray of water appear to be optimum. It is also desirable to avoid having the water under very high pressure or the spray source close to the panel.

One possible source of a subjective error lies in the sketching of the pattern of oil-covered areas disclosed by the condensation of the spray of water. Comparison of the values obtained with three different operators, on a number of occasions, indicated that this error was rather small, being less than 2%. For operators who might have difficulty in this evaluation, it is possible to provide assistance in the form of a viewing screen divided into 100 squares or some similar arrangement.

Some reproducibility data, obtained on different days over a period of several months, are listed in Table III. The values for cleaning index fall well within the range to be anticipated from the magnitude of the standard deviation.

Appreciation is expressed to Lt. Col. C. H. Greenall, officer-in-charge, Maj. W. W. Culbertson, research officer, C. C. Fawcett, associate director, and E. R. Rechel, chief of the Chemical Research Section of Frankford Arsenal Laboratory, as well as the Ordnance Department, for permission to publish this paper. Special thanks are due J. W. Mitchell for his helpful review of the paper.

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Presented at the spring meeting of the Philadelphia Section and the Meeting-in-Print of the American Chemical Society, Division of Analytical and Micro Chemistry, 1945.

Fluorometric Attachment for the Beckman Spectrophotometer

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A fluorometric attachment for the Beckman spectrophotometer for use in the measurement of fluorescence in solutions consists of a light-tight cell compartment equipped with suitable filters and a lamp housing. It uses the receiving and amplifying systems of the Beckman instrument. General Electric B-H-4 mercury lamp is light source. Lamp emission is controlled by Sola constant-voltage transformer No. 30,852 and by proper ventilation of lamp housing.

MEASUREMENT of the fluorescence of solutions as an analytical procedure has been confined primarily to the field of vitamin chemistry, and for this reason, most commercial fluorometers have been designed for use with the brilliantly fluorescing solutions encountered in this field. For weakly fluorescing solutions, such as those encountered in the determination of beryllium (*2*), the commercial instruments at hand proved to be insufficiently sensitive for an accurate measurement. Since a Beckman spectrophotometer was available, it was decided to adapt it for use with these solutions.

The receiving and amplifying system of the Beckman instrument (*1*) possessed the desired characteristics with respect to sensitivity and range, and consequently it could be used with a minimum of change. All that was needed to make it operative was the addition of a source of ultraviolet light and a compartment for holding the optical cell and filters.

An attachment which consisted of a light-tight cell compartment and a ventilated lamp housing was built from sheet brass. The interior of the cell compartment was painted with a nonfluorescent flat black paint. The principal parts are shown in Figures 1 and 2. The original cell compartment was removed from the Beckman instrument and replaced by the attachment, the cell compartment of which was bolted to the original phototube housing. The arrangement is shown in Figure 1, in which 1 is the lamp housing, 2 the cell compartment, 3 the phototube housing, 4 the Beckman spectrophotometer, 5 the ventilating fan, and 6 the ventilating louvers. The ventilating louvers occupy two of the outer adjacent sides and the top of the lamp housing section. An opening is provided on the third side to permit a connection with the fan. Figure 2 is a plan view (covers removed) of the fluorometric attachment and shows the relative positions of the various parts of the instrument.

The light source, a General Electric B-H-4 mercury lamp, was chosen because previous experience had shown it to be generally satisfactory. Another possible light source, the hydrogen discharge tube furnished with the Beckman instrument, was tried on the beryllium-quinizarin solutions and proved unsatisfactory because of the very low intensity of the exciting wave lengths. On the other hand, the B-H-4 lamp because of its strong emission at 3650 Å. is an excellent light source and provides an intense radiation of the proper wave lengths for an efficient excitation of fluorescence in solutions of the type under consideration.

The emission of the mercury lamp is influenced by the voltage and ambient temperature of the lamp; therefore, to ensure constant emission, both voltage and temperature regulation on the lamp were found necessary. For the former, a Sola constant-voltage transformer No. 30,852 specifically designed for use with the H-4 lamp was employed. For the latter, an Eastman darkroom ventilating fan, Model A, was used.

Table I. Stability

Input Voltage	Reading for 22.4 Micrograms of BeO in 25 Ml. of Solution	Reading for 11.2 Micrograms of BeO in 25 Ml. of Solution
110	98.0	46.8
120	101.4	49.0
130	103.8	50.0
Spread, 20 volts	5.8 scale divisions	3.2 scale divisions

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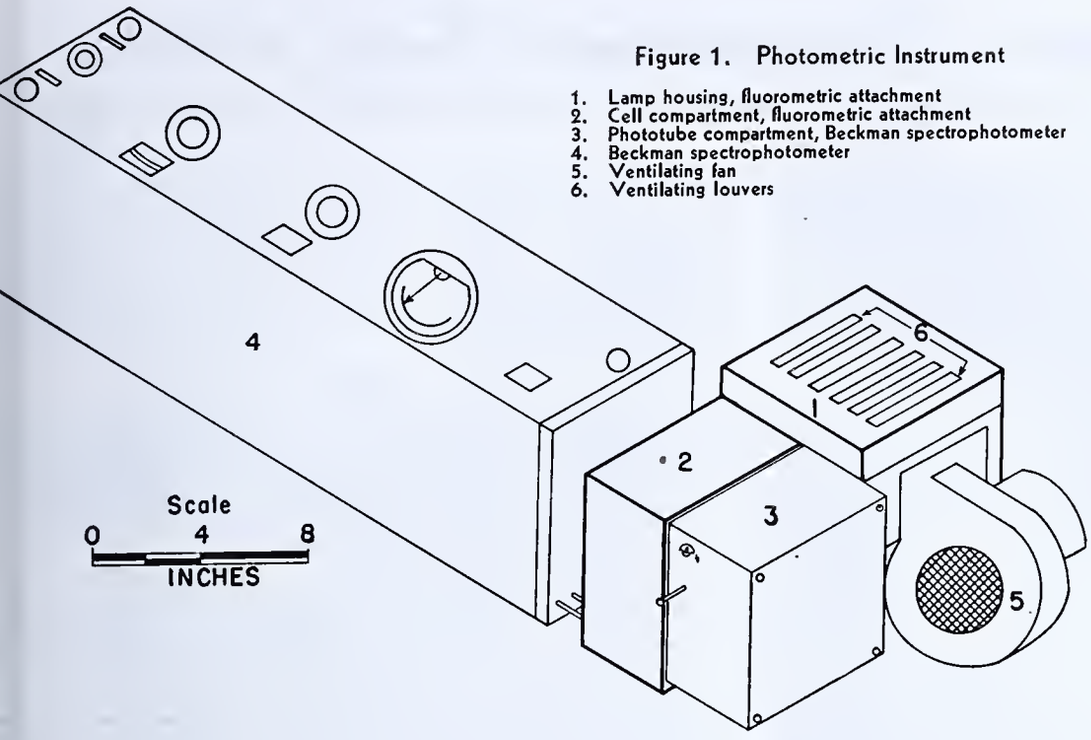

Figure 1. Photometric Instrument

1. Lamp housing, fluorometric attachment
2. Cell compartment, fluorometric attachment
3. Phototube compartment, Beckman spectrophotometer
4. Beckman spectrophotometer
5. Ventilating fan
6. Ventilating louvers

The stability of the instrument accompanying large changes in input voltage is illustrated by Table I. These data were obtained by varying the input voltage to the Sola transformer by means of an autotransformer connected to the line, and noting the change in the phototube response to two of the beryllium-quinizarin standards.

In the case of the stronger solution, a difference of 5.8 scale divisions resulted from a change of 20 volts. This change would correspond to 0.81 microgram of beryllium oxide (value obtained from working curve, micrograms of BeO versus Beckman scale reading), which is 3.6% of the amount measured, or an error of 0.18% per volt. For the weaker solution, the difference of 3.2 scale divisions corresponds to 0.90 microgram of beryllium oxide, which is 8.1% of the amount present, or an error of 0.4% per volt. These figures are given merely to show the performance of the setup under extreme conditions and do not represent the results obtained in normal operation. There was no measurable lag between voltage change and change in reading.

Proper ventilation of the lamp housing provides satisfactory control for the lamp temperature, and equilibrium is generally reached after a half-hour warm-up period.

In all fluorometers certain problems of light filtration exist. Visible light should be excluded from the cell compartment, and ultraviolet light should be excluded from the phototube, yet the fluorescent light must pass freely to the phototube.

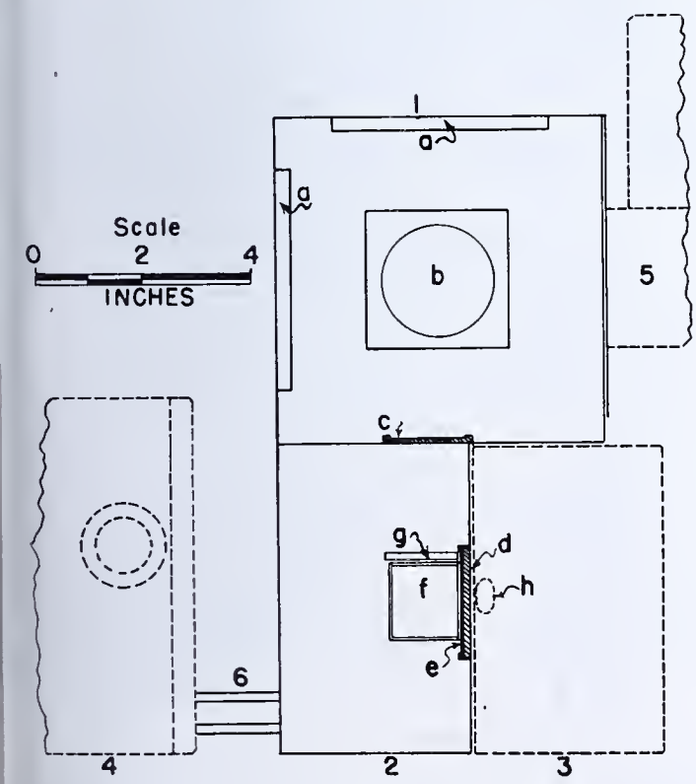

Figure 2. Fluorometric Attachment

1. Lamp housing
   - a. Louvers
   - b. B-H-4 mercury lamp
   - c. Filter for absorbing visible light
2. Cell compartment
   - d. Filter for absorbing ultraviolet light
   - e. Brass diaphragm
   - f. Optical cell
   - g. Guide for optical cell
3. Phototube housing
   - h. Phototube
4. Beckman spectrophotometer
5. Fan
6. Electrical cables

In the present case the combination of filters chosen (Corning Nos. 5874 and 3486), together with the blue sensitive phototube, have the characteristics necessary to permit measurement of the fluorescence. The primary filter (Corning 5874) eliminates all light lying between 4100 and 7000 Å. from the exciting radiation; and the secondary filter (Corning 3486) absorbs all light of a wave length less than 5000 Å. but has a high transmission in the region 5700 to 6400 Å. The phototube is insensitive to light with a wave length greater than 7000 Å. Hence, since the fluorescent radiation is the only light transmitted which affects the phototube, it alone is measured.

The attachment herein described was designed for a particular purpose, the measurement of a weak fluorescence of 5700 to 6400 Å. In this case, the optical glass cell used to contain the solutions was a 31-mm. cube, inside measurement, with a wall thickness of 1 mm. Cells of this type may be purchased from Pyrocell Manufacturing Co., 207 East 84th St., New York 28, N. Y.

Comparative data on the performance of the instrument are given elsewhere (*3*). It can be adapted for use in the measurement of other wave lengths and stronger fluorescence by changing the sensitivity setting of the Beckman instrument, by the proper selection of filters, by varying the size of the aperture in the brass diaphram, and by the use of other light sources. The attachment should prove useful to any laboratory that has a Beckman spectrophotometer in its possession.

ACKNOWLEDGMENTS

The study reported in this paper was under the general direction of J. B. Zadra, chief, College Park Division, Metallurgical Branch, U. S. Bureau of Mines. The writers are indebted to Alton Gabriel and Morris Slavin for assistance in the writing of this paper, to Rudolf Kudlich and his men in the shop for the construction of the attachment, to John P. Wintermoyer and Rebecca Bland for the drawings, and to Stephen L. Windes for assistance with the electrical units.

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Spectrophotometric Determination of Traces of Nickel

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A rapid and precise method of determining traces of nickel in foods and biological materials and in ferrous materials has been developed. The optimum range is 5 to 80 micrograms and in this range the determinations are accurate to ±5%. Nickel is separated from interfering elements by a solvent extraction of the dimethylglyoxime salt of divalent nickel, and is determined by spectrophotometric measurement of the yellow diethyldithiocarbamate complex.

THE accurate determination of traces of nickel in foods and biological material, such as might be introduced into a food product through contact with nickel-bearing corrosion-resistant equipment, is an analytical problem of considerable difficulty. The determination of small amounts of nickel in mild steel is also a time-consuming procedure, since for accurate determination a preliminary separation by means of ether extraction is usually required.

The potassium salt of dithiooxalic acid (*6*) has been used as a colorimetric reagent for nickel. The reagent is specific and sensitive, but the reagent itself as well as the nickel complex is unstable. The literature contains a number of references to the use of dimethylglyoxime as a colorimetric reagent for the determination of nickel (*4*, *5*). This reaction between dimethylglyoxime and nickelic (IV) ion is somewhat empirical, and the results depend upon adherence to a closely defined operational procedure. A number of other reagents have been proposed, but have little to recommend them for general analytical purposes (*1*, *3*).

The method described herein employs two reactions of nickel, both of which have been previously reported. Nickel is first separated by means of a chloroform extraction of the divalent nickel salt of dimethylglyoxime (*5*). After decomposition with dilute hydrochloric acid, and extraction of the nickel from the chloroform, the nickel is converted to the diethyldithiocarbamate complex and extracted with isoamyl alcohol. The nickel is determined in this solution by spectrophotometric measurement of the concentration of the yellow-green nickel complex. This reaction has been widely recognized, but has not to the authors' knowledge been used to determine nickel (*2*).

DISCUSSION OF METHOD

Dimethylglyoxime has long been regarded as one of the most specific organic precipitants available in trace metal analysis. This specificity as a precipitant also characterizes the chloroform extraction described in this report. According to available literature references to the use of sodium diethyldithiocarbamate (*2*) the metals giving colored precipitates soluble in organic solvents are nickel, copper, cobalt, bismuth, and iron. Iron does not react in ammoniacal citrate solutions and nickel is separated completely from the other potential interfering elements by the glyoxime extraction. Copper if present in high concentrations may accompany the nickel to some extent, but may easily be removed from the chloroform solution by an intermediate wash with dilute ammonia.

The nickel dithiocarbamate complex absorbs most strongly in the very edge of the visible spectrum, and since the eye does not adequately resolve minor differences in the concentration of the faintly colored solutions the use of a spectrophotometer is almost mandatory. This low visual sensitivity probably explains in part why the reagent has not been used previously. The data presented in this report were obtained with a Coleman Model 11 Universal spectrophotometer. The curves relating concentration to the logarithm of the transmission are curved not because the solutions fail to obey Beer's law, but because the optical system of the instrument does not produce light beams that are spectrally pure. This instrumental imperfection is much more pronounced at the lower wave length range where the low intensity of the tungsten filament and low sensitivity of the photronic cell both tend to emphasize the effect of scattered light of higher wave length.

REAGENTS

A. AMMONIUM CITRATE, 20% w/v. Dissolve 200 grams of diammonium citrate in 600 ml. of water, adjust to pH 9.0 to 9.5 with ammonium hydroxide, and transfer to a 1-liter separatory funnel. Add 10 ml. of dimethylglyoxime solution (reagent C) and make three extractions with 30-ml. portions of chloroform. Filter the solution and dilute to 1 liter.

B. AMMONIUM CITRATE, 20% w/v. Dissolve 200 grams of diammonium citrate in 600 ml. of water, adjust to pH 9.0 to 9.5 with ammonium hydroxide, and transfer to a 1-liter separatory funnel. Add 10 ml. of sodium diethyldithiocarbamate solution (reagent D) and extract with 20-ml. portions of carbon tetrachloride until the carbon tetrachloride is colorless. Vigorous agitation is required. Add 5 ml. more of carbamate reagent and again extract with carbon tetrachloride. If the solvent layer is colorless the extraction is complete; if it remains yellow, add more carbamate and continue the extraction.

C. DIMETHYLGLYOXIME, 0.1%. Dissolve 0.25 gram of reagent grade reagent in 50 ml. of 95% ethanol and dilute to 250 ml.

D. SODIUM DIETHYLDITHIOCARBAMATE, 0.2%. Dissolve 1 gram in 100 ml. of distilled water, filter, and dilute to 500 ml.

E. HYDROCHLORIC ACID, 0.5 *N*. Dilute 40 ml. of hydrochloric acid (sp. gr. 1.18) to 1 liter with redistilled water.

F. NICKEL STANDARD. Dissolve 0.500 gram of pure nickel in 20 ml. of (1 + 1) nitric acid and dilute to 1 liter. Prepare a working standard by diluting 10 ml. of this solution to 1 liter with 0.5 *N* hydrochloric acid. One milliliter of the working standard contains 5 micrograms of nickel.

PREPARATION OF SAMPLES

FOOD PRODUCTS AND BIOLOGICAL MATERIALS. Transfer a representative sample estimated to contain 5 to 100 micrograms of nickel to a 250-ml. Erlenmeyer flask (as much as 25 grams of most foods may be satisfactorily handled). Dry the sample on a steam bath, in an air oven, or on a low-temperature hot plate. Add 20 to 25 ml. of nitric acid and heat gently to begin the oxida-

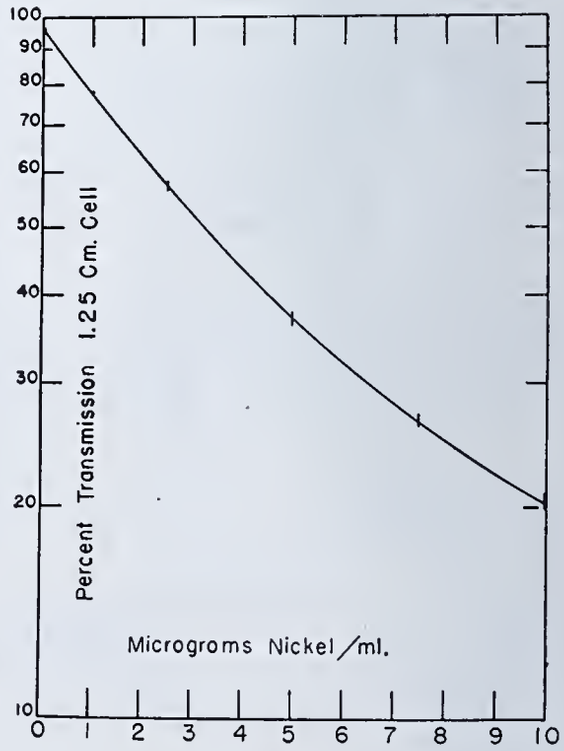

Figure 1

Table I. Recovery of Nickel from Iron Solutions

Iron Added	Impurity Added	Nickel Added	Nickel Found	Recovery
Gram	*Mg.*	γ	γ	%
0.20	...	2.5	2.7	108
		5.0	4.9	98
		7.5	7.9	105
		10.0	10.0	100
		12.5	12.1	97
		15.0	14.8	99
0.10	...	25.0	25.7	103
		50.0	48.6	97
		75.0	72.3	97
		100.0	97.1	97
0.20	Co 10	25	25.2	101
	Bi 10	25	25.6	102
	Cu 10	25	25.1	100

Table II. Recovery of Nickel from Tomato Catsup

Nickel Added to 10-Gram Samples of Catsup	Nickel Found	Nickel Present	Recovery
γ	γ	γ	%
0	16.5	(16.5)	...
0	16.5	(16.5)	...
10	26.9	26.5	102
25	40.0	41.5	94
50	66.0	66.5	99
65	80.0	81.5	98
80	94.4	96.5	98

tion. When the initial reaction subsides, add 5 ml. of sulfuric acid and continue heating. Add more nitric acid in small increments as the mixture darkens and begins to char. When fumes of sulfur trioxide are evolved with no further charring or darkening, add 1 ml. of perchloric acid and continue heating until the perchloric acid has been volatilized and sulfuric acid is refluxing freely. Allow to cool and dilute with 20 to 25 ml. of distilled water.

STEELS. Dissolve 0.200 gram of steel in a small covered beaker with 15 ml. of nitric acid (1 + 2). After the first vigorous reaction subsides, add 10 ml. of perchloric acid and heat slowly until the perchloric acid is refluxing freely and solution of the steel is complete. Cast iron samples may require the addition of more nitric acid to complete the oxidation and solution of refractory carbides. Cool the digest and dilute with water. If the nickel content of the steel is expected to run over 0.04%, dilute to volume and use an aliquot containing 20 to 80 micrograms of nickel.

ISOLATION OF NICKEL

Transfer the prepared sample, or a suitable aliquot, to a 125-ml. separatory funnel, and add 10 ml. of citrate buffer (reagent A) and water to make the volume approximately 40 ml. Make alkaline to phenol red (pH 8.5 to 9.0) with ammonium hydroxide, and add 5 ml. of dimethylglyoxime (reagent C) and 10 ml. of chloroform. Stopper the flask securely and shake vigorously for 1 minute. Drain all but a few drops of the solvent layer into a second funnel containing 25 ml. of dilute ammonium hydroxide (1 + 50). Repeat the extraction with an additional 5-ml. portion of chloroform, and drain this too into the second funnel, drawing off the solvent as completely as possible without introducing any of the aqueous phase. Shake the combined solvent extracts and dilute ammonium hydroxide for 1 minute, allow the phases to separate cleanly, and draw off the aqueous layer through a pipet attached to a vacuum line. Wash down the sides of the funnel with distilled water, and again remove the aqueous layer.

Add 25 ml. of 0.5 *N* hydrochloric acid (reagent E), shake vigorously for 1 minute, and allow to separate. Drain off and discard the solvent. Add a few milliliters of carbon tetrachloride, shake briefly to extract dissolved chloroform, and draw off the solvent as completely as possible, being careful to dislodge and remove the drop which usually floats on the surface.

DETERMINATION OF NICKEL

To the acid solution add 5 ml. of citrate buffer (reagent B). (The two citrate solutions should not be used interchangeably. If solution B, which contains residual carbamate, were used in the first extraction, copper, cobalt, lead, zinc, cadmium, bismuth, and possibly other metals would be partially extracted with the nickel. If solution A were used in the final extraction, impurities in the citrate such as copper and cobalt which are not removed by the dimethylglyoxime extraction would contaminate the final extract.) Add a small piece of litmus paper and make slightly alkaline with ammonium hydroxide, adding 8 to 10 drops in excess. Add 10.0 ml. of isoamyl alcohol and 5 ml. of carbamate solution (reagent D), stopper the funnel, and shake vigorously for at least 2 minutes. Draw off the aqueous phase, transfer the solvent layer to the spectrophotometer cell, and determine the transmission at a wave length of 385 mu. The cell may be placed momentarily in hot water in the event that the solution becomes cloudy due to separation of dissolved water.

PREPARATION OF STANDARDS

To a series of 125-ml. separatory funnels, add 0, 1, 2, 3, 5, 7, 10, and 15 ml. of the working standard solution, and dilute to 25 ± 0.5 ml. with 0.5 *N* hydrochloric acid. Treat these standards exactly as described under the preceding section, beginning with the addition of the citrate buffer (reagent B).

Although 385 mu is given as the wave length of minimum transmission, the actual value may vary somewhat from one instrument to another, depending upon the width and spectral purity of the wave-length band isolated. Figure 1 shows an average standard curve prepared from data obtained over a period of several weeks. The vertical lines intercepted by the curve indicate the spread of some 10 to 12 values in each case. These standards were not all prepared with the same lot of reagents and consequently the data reflect variations in reagents as well as instrumental and manipulative errors. Although this curve is reproducible over relatively short periods of time, some variation may be expected due to the aging of the tungsten filament bulb and the accompanying change in spectral emission.

The sensitivity and precision of the instrumentation could be materially increased through the use of a spectrophotometer giving greater dispersion and more nearly monochromatic light. Somewhat greater sensitivity and precision would also be gained if it were possible to use spectrophotometer cells designed to permit transmission measurements on small volumes of solution at longer cell lengths.

Table III. Recovery of Nickel from Evaporated Milk

Nickel Added to 10-Gram Samples of Evaporated Milk	Nickel Found	Nickel Present	Recovery
γ	γ	γ	%
0	0.2	(0.2)	...
0	0.2	(0.2)	...
0	0.3	(0.2)	...
5	5.2	5.2	100
10	10.3	10.2	99
15	15.4	15.2	101
30	30.3	30.2	100
50	49.8	50.2	99

RECOVERY DETERMINATIONS

Known amounts of nickel were added to aliquots of a nickel-free iron solution containing 0.10 and 0.20 gram of iron, to 10-gram samples of tomato catsup, and to 10-gram samples of evaporated milk. These samples then were subjected to the procedure described. The data presented in Tables I, II, and III show that satisfactory recovery of added nickel is obtained, even in the presence of relatively large amounts of potentially interfering elements.

In order to test further the application of the method, a number of Bureau of Standards steel samples were analyzed for nickel

Table IV. Determination of Nickel in Bureau of Standards Samples

N.B.S. Sample No.	Reported Nickel Content of Standard Samples: Certified value	Range of results of collaborating analysts	Nickel Found
10 d	0.002	0.001–0.004	0.0054
8 g	0.011	0.010–0.013	0.0099
55 a	0.019	0.019–0.020	0.0186
15 d	0.045	0.022–0.026	0.0233
11 e	0.036	.....	0.0446
74	0.227	0.034–0.039	0.0363
20 d	0.152	0.217–0.239	0.231
21 c		0.144–0.17	0.149

by the method described. The results of these determinations (Table IV) are in very good agreement with the certified value, when the variation in the values reported to the Bureau of Standards by the collaborating analysts is taken into consideration.

SUMMARY

A colorimetric micromethod for nickel is described which is applicable to the determination of traces of nickel in foods and biological materials as well as in carbon steels. It is based upon the isolation of nickel by solvent extraction of the dimethylglyoxime complex and the subsequent determination by means of sodium diethyldithiocarbamate. Recovery determinations and analyses of standard samples indicate that nickel may be determined with satisfactory precision when present in amounts of 1 microgram or greater.

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Amperometric Microtitration of Very Dilute Chromate Solutions

Using the Rotating Platinum Electrode

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Chromate (chromic acid) can be titrated rapidly by amperometric titration with ferrous iron, using the rotating platinum wire microelectrode as the indicator electrode. The potential of the indicator electrode is maintained at 1.0 volt *vs.* the saturated calomel electrode which is used as the reference electrode. The method is accurate and precise to within 0.5% at concentrations as small as 1 to 2 × 10^{-4} *M* chromate. The method can also be used in the reverse titration of traces of ferrous iron with dichromate.

CHROMATE in very dilute acid solutions can be accurately determined by amperometric titration with ferrous sulfate, using a rotating platinum electrode as indicator electrode. This method does not involve an indicator correction; this latter is appreciable when very dilute solutions are titrated. The principles and technique of amperometric titrations with the rotating platinum wire microelectrode have been discussed and described by Laitinen and Kolthoff (*1*, *2*). The method described here for chromate involves the titration of an acidified chromate solution with a ferrous iron solution. The end point is found graphically as the point of intersection of the "residual current" line before the end point and of the anodic diffusion current line of the ferrous iron after the end point. Under the experimental conditions used in this work the residual current was zero.

The anodic oxidation of ferrous iron to ferric gives a well-defined diffusion current at a rotating platinum microelectrode. Current-voltage measurements using 5 × 10^{-5} *M* ferrous ammonium sulfate in 0.1 *M* perchloric acid are shown in curve 1, Figure 1. In curve 2, the residual current of 0.1 *M* perchloric acid solution is shown. At a potential of +1.0 volt of the rotating electrode, as measured against a saturated calomel reference electrode, proportionality was found between the concentration of the ferrous ammonium sulfate and the diffusion current, as may be seen in Table I.

At potentials of +1.0 volt no current is obtained by electrode reactions with Cr^{++++++}, Cr^{+++}, or Fe^{+++}. Thus when an acid solution of chromate is titrated with a ferrous ammonium sulfate solution no current is observed at a rotating platinum electrode potential of 1.0 volt until all the chromate has been reduced and the solution contains an excess of ferrous iron. By measuring the current after adding successive amounts of ferrous solution and obtaining two or more values in the presence of an excess of ferrous solution the end point is found by graphical extrapolation to zero current. The volume of ferrous solution at that point is equal to that at the equivalence point. Typical titrations are shown in Figure 2.

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PROCEDURE

The chromate solution to be titrated is placed in a beaker of suitable size and a rotating platinum microelectrode and salt bridge are placed in position. A saturated calomel electrode is used as an outside reference electrode. If the solution is not already acid, sufficient acid is added to give a concentration of 0.1 *M* (perchloric, hydrochloric, sulfuric, or nitric acid may be used). A 1-volt difference of potential is applied across the platinum (anode) and saturated calomel electrodes, using a salt bridge of low resistance. A 0.01 *N* standard ferrous ammonium sulfate, 0.05 *M* in sulfuric acid, is added to the chromate solution from a microburet until a current is observed on the microammeter or other suitable current-indicating instrument. The sides of the beaker are rinsed with distilled water and the diffusion current of

Table I. Diffusion Currents of Ferrous Ammonium Sulfate Solutions in 0.1 M Perchloric Acid Solution

(Rotating platinum electrode. Potential + 1.0 volt *vs.* saturated calomel electrode)

Molar Concentration of Ferrous Iron × 10^5	Diffusion Current, Microamperes	i_d/c × 10^5
10	8.9	0.89
5	4.5	0.90
2	1.8	0.90
1	0.9	0.90

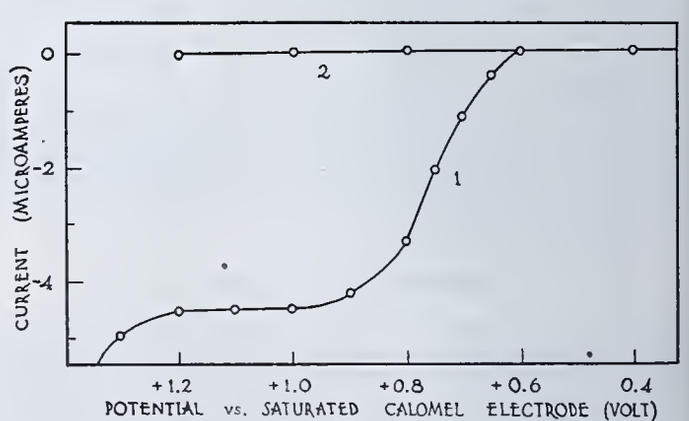

Figure 1. Current-Voltage Curve of 5 × 10^{-5} M Ferrous Ammonium Sulfate in 0.1 M Perchloric Acid

1. 5 × 10^{-5} *M* ferrous ammonium sulfate in 0.1 *M* perchloric acid
2. 0.1 *M* perchloric acid

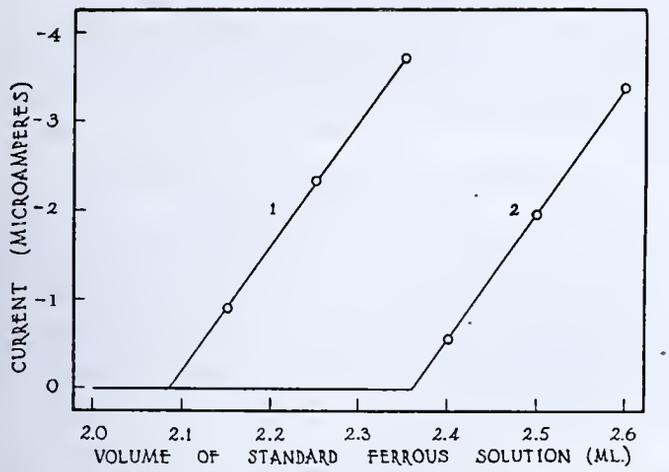

Figure 2. Amperometric Titration of Potassium Chromate in 0.1 *M* Perchloric Acid with 0.00962 *N* Ferrous Ammonium Sulfate

1. 50 ml. of 1.333 × 10^{-4} *M* chromate
2. 50 ml. of 1.510 × 10^{-4} *M* chromate

the ferrous iron is measured. The current is measured again after the addition of 0.2 ml. more of ferrous solution. One or two more readings are made after successive addition of more ferrous iron. On graph paper, points representing the microamperes (ordinate) and volume of ferrous solution (abscissa) are plotted. These points lie on a straight line. The volume of ferrous solution at zero current is obtained by drawing a line through these points. For the most accurate work the current should be corrected to compensate for dilution (*2*).

Several dilute chromate solutions were analyzed in this manner. The ferrous solution used was prepared by diluting a 0.098 *N* ferrous ammonium sulfate solution tenfold. The diluted solution was standardized against potassium chromate, using sodium diphenylamine sulfonate as indicator and correcting for the indicator blank. The concentration found was 0.00961 *N*. In Table II are the results obtained in the amperometric titration of dilute chromate solutions with 0.0096 *N* ferrous ammonium sulfate solution. The solutions were 0.1 *N* in perchloric acid.

Table II. Amperometric Titration of 50 Ml. of Dilute Chromate Solutions

Molar Concentration of Chromate × 10^4	Volume of 0.01 *N* Ferrous Solution, Ml.	Calculated Molar Concentration of Chromate Solutions × 10^4	Error, %
1.00	1.560	0.999	−0.1
1.333	2.086	1.336	+0.2
1.510	2.358	1.511	+0.1
1.667	2.603	1.668	+0.1
1.667	2.590	1.660	−0.4
2.000	3.122	2.000	0.0
2.000	3.120	1.999	−0.1

The rapid method, which is accurate and precise to 0.5% even at concentrations as small as 1 to 2 × 10^{-4} *M* chromate, can also be used for the reverse titration of traces of ferrous iron with dichromate. The current decreases continuously during the titration and becomes zero at the end point. Again the exact location of the end point is found graphically.

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Rapid Estimation of Rubber in Guayule Latex Dispersions

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SPENCE (*4*) has shown that the rubber of guayule (*Parthenium argentatum* Gray) is recoverable as a latex which yields a product of high purity and superior quality. His observations on the quality of the latex rubber have been confirmed recently by Clark and Place (*1*). The occurrence of the latex in small separate cells rather than in a continuous duct system makes necessary a fine comminution of the shrub, and as a result the dilute dispersions initially obtained are highly contaminated with nonrubber plant and soil fragments. A microscopic method for the rapid estimation of rubber in these dispersions has been developed.

The microscopic method, which consists essentially in the identification and counting of rubber latex particles in a small volume of dispersion, is advantageous in several respects. It requires a minimum amount of equipment and is rapid, taking from 10 to 20 minutes for a determination. A rapid method is important because of the instability of some dispersions and the need of knowing the approximate concentration of dispersed rubber during a recovery experiment. Furthermore, only a small quantity of dispersion, as little as one drop, is required for the microscopic method, and it is possible to distinguish between dispersed and partly agglomerated latex in the samples. In contrast, the chemical methods of analysis (*5*, *7*) are time-consuming, do not distinguish between dispersed and partly agglomerated latex, and require larger samples and more extensive equipment.

STANDARDIZATION AND PROCEDURE

The method is standardized by making counts on a number of guayule latex dispersions of known rubber content, as determined by duplicate analyses by the tetrabromide method (*7*). In this method, the dispersions are extracted in a Waring Blendor with benzene, and rubber hydrocarbon is then determined gravimetrically by precipitation as the tetrabromide. Only dispersions free from agglomerated or partly agglomerated rubber particles should be used for the standardization. Since some dispersions are unstable, the microscopic counts should be made at the time the chemical analyses are begun.

A small sample (usually 1 ml.) of a dispersion of known rubber content is mixed with neutral distilled water to give a known dilution in which the rubber latex particles can be accurately counted in a Petroff-Hausser bacteria counter. Probably a hemacytometer would serve as well, although such a chamber was not tested in the present case. Owing to the tendency of the latex particles to accumulate at the surface on standing, the dispersion must be thoroughly agitated, not only just before sampling for dilution but also after dilution, immediately before mounting in the counter. A dilution which gives 2 to 5 recognizable latex particles per square (1/20,000 cu. mm.) has been found most satisfactory. To ensure a uniform film of dispersion for the mount, the usual precautions must be taken, such as carefully adjusting the cover glass and blotting the excess liquid.

For one determination, all identifiable rubber latex particles, irrespective of size (usually 0.4 to 3.5μ in diameter), in 36 squares in each of two mounts are counted. If agreement between the number of particles counted in the two mounts is not within 5%, counting should be continued on a third mount, or on an additional number of mounts until such agreement is obtained. The count is readily made with a magnification of approximately 800 to 900 diameters, such as is obtained with a 4-mm. objective and 20× ocular. Each particle must be brought into clear focus for identification; those too small for identification should not be counted. Since all flow of particles must be eliminated during counting, it is sometimes necessary to readjust the cover glass

Table I. Standardization of Microscopic Method for Estimation of Rubber in Guayule Crude Latex Dispersions

(N, average number of latex particles per square; D, dilution factor; C_0, concentration of rubber in original dispersion as determined by chemical method; $k = C_0/ND$; C, concentration of rubber in original dispersion calculated from relation $C = 1.85\ ND$.)

Original Plants			Extracted Latex Dispersions					
						Rubber		
Description[a]	Resin[b], %	Rubber[b], %	N	D	k	Microscopic, C	Chemical, C_0	Difference, %
						Mg./100 ml.		
YW	...	3.3	3.00	10	1.77	56	53	+5.7
YX	5.9	8.2	2.42	25	1.87	112	113	−0.9
YX	...	7.3	2.87	25	1.87	133	134	−0.7
YX	6.0	8.3	3.67	25	1.84	170	169	+0.6
YX	5.9	8.2	4.08	25	1.93	189	197	−4.1
YX	...	7.3	2.75	50	1.79	254	246	+3.3
YW	6.8	3.6	2.75	50	1.99	254	273	−7.0
YW	6.8	3.6	3.42	50	1.93	316	330	−4.2
YX	5.4	5.2	2.08	100	1.74	385	361	+6.7
MW	9.0	15.0	2.17	100	1.94	401	421	−4.8
YX	5.6	7.7	2.79	100	1.87	516	521	−1.0
YX	5.4	8.7	3.50	100	1.81	648	632	+2.5
YX	6.8	8.6	3.54	100	1.96	655	694	−5.6
YX	...	8.0	3.92	100	1.82	725	715	+1.4
YX	...	8.0	4.25	100	1.83	786	779	+0.9
MW	9.0	15.0	4.83	100	1.89	894	911	−1.9
YX	5.4	8.7	2.67	200	1.78	988	947	+4.3
YX	...	8.0	2.58	1000	1.74	4773	4501	+6.0
				Av.	1.85			±3.4

[a] Y, 2-year old plants; M, 12-year old plants. X, defoliated plants; W, whole or nondefoliated plants. Data in first three columns indicate range in applicability of method rather than any relationship between original rubber content and amount of rubber extracted.

[b] Moisture-free basis.

carefully. Often slight pressure on the margin of the cover glass will suffice. The predominantly spherical rubber latex particles can readily be distinguished from the variously shaped and colored extraneous nonrubber matter, such as cell-wall fragments, protoplasmic granules, chloroplasts, soap particles, bacteria, and silt. If difficulty is experienced initially in identifying the rubber particles, a portion of them may be stained on a separate slide with a rubber stain (*6*). Staining, however, coagulates the particles.

Standardization of the method is illustrated by Table I. The observed average number of particles per square, N, in the diluted dispersion, the dilution factor D, and the rubber content of the undiluted dispersion, C_0, the latter determined by the chemical method of analysis (*7*), are shown in columns 4, 5, and 8. Constant k in column 6 is the ratio of the concentration of rubber in the diluted dispersion (C_0/D) to the average number of particles per square, or $k = C_0/ND$. The average value of k for a number of dispersions, as shown in the table, was 1.85. Thus the standardization of the method for the guayule latex dispersions is expressed by the relation $C = 1.85\ ND$.

The quantity of rubber in an unknown dispersion is determined microscopically by counting the particles in an appropriate dilution of the dispersion in a manner identical with that employed during standardization. The concentration of rubber in the original dispersion, in mg. per 100 ml., is calculated by the equation $C = 1.85\ ND$.

DISCUSSION

The concentration of rubber calculated from the microscopic count (column 7) using the relation $C = 1.85\ ND$ and the concentration determined by the chemical method (column 8) are tabulated in Table I. The average per cent difference between the two methods (±3.4) is sufficiently small to establish the validity of the microscopic method. In practice, in some cases where the results obtained by the two methods differed rather widely, a repeated chemical analysis showed the results of the original chemical analysis to be in error. In other cases, differences in results between the two methods may be due to the fact that the chemical method, unlike the microscopic, does not differentiate between dispersed and agglomerated rubber.

The error produced by failure to count the submicroscopic rubber particles is negligible, since these particles, although numerous, constitute only an insignificant fraction of the total rubber. Studies in this laboratory of electron micrographs of kok-saghyz latex show that only 2% of the total volume of all particles occurs in particles smaller than 0.3μ in diameter, although by number these particles constitute 87% of the total. Similar results were obtained by Lucas (*3*) on hevea latex. The authors' findings and those of Spence (*4*) indicate that the rubber latex particles of guayule are larger than those of kok-saghyz and hevea.

It would be possible to standardize the microscopic method independently of the chemical method if the average particle mass and the proportion of rubber hydrocarbon in the particles were known. No complete standardization of this sort was attempted in the present case. As a test of its feasibility, however, a preliminary study of the particle size distribution was made visually by means of a micrometer eyepiece. The average particle mass was 9.9×10^{-13} gram. This figure was calculated after measuring the diameters of 600 particles and assuming that they were spheres with a density of 0.92 (*2*).

The concentration of rubber in a dispersion is expressed, as before, by $C = kND$, but now the constant k is equal to Apm, where m is the average particle mass, p is the proportion of rubber hydrocarbon in the particles, and A is a constant 2×10^{12} dependent only on the units and on the volume used for determining N. In the present case the value calculated for k is 1.98 if p is assumed to be unity (100% rubber hydrocarbon), or 1.73 if p is assumed to be 0.875, a value postulated by Kemp (*2*) for hevea particles. These values of k are in good agreement with the value 1.85 found by standardization in terms of the chemical method. The results indicate that an accurate standardization could be carried out which would be independent of any chemical method except for a determination of p.

The wide applicability of the microscopic method to various types of guayule latex dispersions is illustrated by the data of Table I and by subsequent analysis of many samples, in which frequent comparisons with the chemical method were made. Dispersions examined included those extracted in various aqueous media from 2- and 12-year old plants, from whole and defoliated shrub, from fresh and stored plants, and from plants of both high and low resin and rubber content. Agreement with the chemical method under these varying conditions indicated that the rubber latex particles were readily distinguishable from any other particles present, and further that the size distribution and composition of these rubber particles were remarkably constant. On storage after extraction, however, the particles may change in size and possibly composition.

The microscopic method of analysis has been employed in various types of research on guayule. By means of small-scale experiments in which pieces of living guayule tissue were ground in a mortar in anticoagulant solutions and the resulting latex dispersions were analyzed microscopically, significant information on the distribution of latex in the plant and on the effect of pH, enzymes, soaking, temperature, surface active agents, storage, etc., on latex extractions has been obtained. The microscopic method has been applied also in evaluating the effectiveness of each of a series of steps in large-scale latex recovery experiments. With appropriate standardization and with suitable extraction of latex, the method may be applied as a rapid quantitative test for rubber in selection, breeding, and recovery work on other rubber-bearing plants. The data of Lucas (*3*), showing that about 90% of the rubber in hevea latex occurs in particles 0.4 μ or larger in diameter, suggest that the method may be useful also in the rapid analysis of hevea latex.

ACKNOWLEDGMENT

Credit is due C. O. Willits of this laboratory for the chemical analyses.

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NATURAL Rubber from Domestic Sources, Paper 12.

Modified All-Dithizone Method for Determination of Traces of Copper

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A modified all-dithizone procedure in which the copper is extracted in Mojonnier fat-extraction flasks and determined by the mixed color method is described. Interference and contamination by likely metallic ions are eliminated. Data on recoveries of added copper in several biological products are given. Recoveries of better than ±0.2 microgram are obtained.

During the examination of dried whole and skim milk for copper content according to army specifications, the need for a rapid, reproducible method adaptable for routine use was recognized. Various procedures based upon the use of sodium diethyldithiocarbamate (*5*, *6*) have been recommended. However, since nickel, cobalt, and bismuth interfere, these methods leave much to be desired. In other methods (*3*, *4*, *8*, *9*), which combine the desirable features of dithizone and sodium diethyldithiocarbamate, the copper is separated from the nickel, cobalt, and part of the bismuth by extraction from acid solution with dithizone. Only copper and part of the bismuth will be carried into the dithizone layer. The dithizonate complexes are destroyed by ashing and the copper is determined finally with the carbamate reagent. Bismuth interferes, causing high results. Greenleaf (*4*), however, eliminated interference from bismuth by extraction with acidified potassium iodide prior to destruction of the dithizonate.

Bendix and Grabenstetter (*1*) describe an all-dithizone method in which the copper is extracted by means of dithizone at a pH of 2.3, along with gold, platinum, palladium, silver, mercury, bismuth, and stannous tin. No other metals react with dithizone at this pH. Gold, platinum, and palladium are not considered because of their rarity; acid digestion oxidizes stannous tin to the stannic state, in which it does not form complexes with dithizone; silver, mercury, and bismuth are removed as complex iodides by extraction with 2% acidic potassium iodide. The excess dithizone is then removed from the carbon tetrachloride solution with an ammonia wash, and the copper determined colorimetrically as the violet-colored keto copper dithizonate.

In the authors' experience this method, though practicable, presents two difficulties: (1) the transfer through three separatory funnels without the hazard of contamination, particularly by stopcock lubricant, and (2) the fact that the ammonia wash changes variable portions of the violet-colored keto copper dithizonate into the yellowish-brown enol tautomer. In this observation the authors have confirmed Sandell's (*7*) earlier reference.

In the method herein presented copper is extracted with dithizone at a pH of 2.3 in a Mojonnier fat-extraction flask or a container of similar design. This flask is so designed that 20 ml. of the heavier carbon tetrachloride solution of dithizone remain in the bulb of the flask when any aqueous solution is poured off above it. The obvious advantage of such a flask in this particular procedure lies in the fact that after each extraction it is the aqueous layer that is discarded. Therefore all extractions can be made in one flask and the reactive dithizone is safely protected from air-, glass-, or stopcock-borne contamination. The carbon tetrachloride layer is then shaken with 2% acidic potassium iodide solution, the copper remaining in the carbon tetrachloride layer as the dithizonate, while the other metals are extracted into the aqueous layer as iodide complexes and discarded. The concentration of copper is then measured in a spectrophotometer by the mixed color method at the wave length of maximum absorption of keto copper dithizonate. The use of the mixed color method of measurement obviates the necessity for removing uncombined dithizone with ammonia, thereby eliminating the possibility of copper loss through enol formation (*7*).

APPARATUS

A spectrophotometer, Coleman, model 6, with cuvettes, No. 6-504B, 19 × 150 mm.

Mojonnier fat-extraction flasks fitted with ground-glass stoppers.

A mechanical shaker, averaging 275 oscillations per minute.

Glassware, cleaned with concentrated nitric acid, and well rinsed with tap, distilled, and redistilled, metal-free water.

REAGENTS

Water, redistilled from Pyrex, for making all solutions.

C.P. nitric acid, redistilled from Pyrex.

C.P. sulfuric acid. Since different lots of sulfuric acid are contaminated by varying microquantities of copper, a large volume of pooled acid is advisable.

C.P. concentrated ammonium hydroxide.

Cresol red indicator solution, 0.02 gram per 100 ml. of redistilled water.

Buffer reagent, pH 2.3. Dissolve 8.3 grams of C.P. anhydrous disodium hydrogen phosphate and 38 grams of C.P. citric acid in redistilled water. Purify by shaking in a separatory funnel with successive portions of the concentrated solution of dithizone, until there is no noticeable change in the color of the dithizone. Wash with C.P. carbon tetrachloride until the washings are water-clear. Transfer to a 250-ml. volumetric flask and dilute to volume with redistilled water; 2 ml. of this reagent will buffer 25 ml. of water at pH 2.3.

Concentrated solution of dithizone (diphenylthiocarbazone), 15 mg. per liter. Dissolve 15 mg. of dithizone in 1 liter of C.P. carbon tetrachloride. This can best be accomplished by first dissolving it in about 10 ml., then diluting to 1 liter. Store the solution in a black-painted bottle in the refrigerator until ready to use.

Dilute dithizone solution, 7.5 mg. per liter. Make a 1 to 1 dilution of the concentrated dithizone with C.P. carbon tetrachloride. Since this solution is extremely unstable, prepare fresh daily and keep in refrigerator unless actually in use. At all times during the determination, shield the solution from direct sunlight.

Two difficulties are encountered in preparation of dithizone solutions. Generally, dithizone as manufactured by Eastman Kodak Co. is satisfactory for use; however, the commercial product may be purified, according to the method outlined by Sandell (*7*). Occasional lots of reagent grade carbon tetrachloride are unfit for use because of the presence of decomposition products, such as phosgene and free chlorine. Methods of reclaiming carbon tetrachloride have not been devised; therefore, a bad lot should be discarded.

The practice of "stripping" the dithizone solution is recommended as a simple daily procedure for determining whether suitable for use. This is performed by shaking 10 ml. of concentrated dithizone solution with 25 ml. of 1 to 200 C.P. ammonium hydroxide; if satisfactory, the heavier carbon tetrachloride layer will be water-clear, or at most, slightly tinged with yellow.

Acidic potassium iodide reagent, 2%. Dissolve 10 grams of C.P. potassium iodide in 450 ml. of redistilled water, acidify with 5 ml. of 1 *N* hydrochloric acid, and add 0.1 *N* sodium thiosulfate, dropwise, to expel any yellow color of free iodine. Wash with dithizone and carbon tetrachloride as in the preparation of the buffer reagent, dilute to 500 ml. with redistilled water, and add a few milliliters of C.P. carbon tetrachloride as a preservative. Store in the refrigerator, inspecting daily for decomposition of the iodide, and adjusting, if necessary, with a drop or two of thiosulfate.

Standard copper solution, stock, 1 mg. of copper per ml. Dissolve 0.5000 gram of electrolytic sheet copper in 20 ml. of 6 *N* nitric acid, and evaporate almost to dryness. Add 2 to 3 drops of glacial acetic acid and transfer the solution quantitatively to a 500-ml. volumetric flask, diluting to volume with redistilled water.

Working standard copper solution, 1 microgram per ml.

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Table I. Recovery of Copper from Solutions Containing Other Metallic Ions

Copper Added	Other Metal Added	Copper Recovered: Without KI shaking	Copper Recovered: With KI shaking	Error
Micrograms	*Micrograms*	*Micrograms*	*Micrograms*	*Micrograms*
	Fe^{+++}			
5.0	50.0	....	4.85	−0.15
5.0	50.0	....	4.90	−0.10
5.0	1000.0	....	4.95	−0.05
5.0	1000.0	....	5.00	0.0
	Sn^{++++}			
4.0	50.0	....	4.10	+0.10
4.0	100.0	....	3.90	−0.10
4.0	200.0	....	3.95	−0.05
5.0	500.0	....	5.00	0.0
5.0	500.0	....	4.80	−0.20
	Pb^{++}			
3.0	30.0	....	3.0	0.0
4.75	1000.0	....	4.80	+0.05
4.75	1000.0	....	4.80	+0.05
6.0	200.0	....	6.0	0.0
5.0	100.0	5.10	5.05	+0.05
5.0	100.0	5.20	5.10	+0.10
	Zn^{++}			
5.0	100.0	5.10	5.00	0.0
5.0	100.0	4.95	4.85	−0.15
3.0	200.0	....	3.00	0.0
4.0	200.0	....	3.90	−0.10
	Cd^{++}			
3.0	200.0	....	3.15	+0.15
4.0	200.0	....	4.05	+0.05
5.0	100.0	5.05	5.00	0.0
	Co^{++}			
5.0	100.0	5.00	5.00	0.0
5.0	200.0	5.00	5.05	+0.05
	Ni^{++}			
5.0	100.0	5.00	4.90	−0.10
5.0	125.0	4.90	5.15	+0.15
	Mn^{++}			
5.0	100.0	5.10	5.00	0.0
5.0	200.0	5.05	4.95	−0.05
	Bi^{+++}			
5.0	50.0	5.10	5.10	+0.10
5.0	500.0	....	5.15	+0.15
5.0	200.0	5.00	5.15	+0.15
	Hg^{++}			
5.0	20.0	....	5.00	0.0
5.0	20.0	....	4.80	−0.20
5.0	30.0	....	4.25	−0.75
5.0	30.0	....	4.20	−0.80
	Ag^{+}			
5.0	20.0	....	4.90	−0.10
5.0	20.0	....	4.95	−0.05
5.0	30.0	....	3.50	−1.50
5.0	30.0	....	4.0	−1.00

Dilute exactly 10 ml. of the concentrated standard to 100 ml. After mixing, dilute exactly 10 ml. of the intermediate solution to 1000 ml. to prepare the working standard, containing 1 microgram per ml.

PREPARATION OF SAMPLE

Weigh and transfer to a 500-ml. Kjeldahl flask a portion of well-mixed food or biological material containing less than 70 micrograms of copper. Add 10 to 15 ml. of C.P. nitric acid, redistilled, and 2 short lengths (about 1.25 cm., 0.5 inch) of copper-free, thin-walled glass tubing to prevent superheating. Add exactly 10 ml. of C.P. sulfuric acid. Heat the contents of the flask over a direct flame, maintaining oxidizing conditions at all times by the dropwise addition of nitric acid. Oxidation is complete when there is no perceptible darkening of the fluid upon the appearance of thick white fumes of sulfur trioxide. The final solution should be colorless, or at most straw-colored. Continue heating about 5 minutes after this stage is reached to assure complete digestion.

Cool the flask, add 50 ml. of redistilled water, and resume heating until sulfur trioxide fumes appear. Repeat the dilution and evaporation in order to remove all oxides of nitrogen. Caution must be exerted at this point to avoid superheating, especially when digesting milk. Use of a hot flame and constant shaking until boiling has begun are helpful. The reappearance of a precipitate is a signal to resume shaking. Transfer the contents of the flask to a 100-ml. volumetric flask, and dilute to volume with redistilled water. Conduct a blank, using 10 ml. of sulfuric acid, and approximately the same quantity of nitric acid as in the sample.

When digesting substances which foam, it is more satisfactory to carry out the heating for some time without the addition of the sulfuric acid. When the oxidation seems to be almost complete, cool the mixture, add 10 ml. of sulfuric acid, and carry the digestion to completion.

PROCEDURE

Pipet a suitable aliquot of the sample solution (containing less than 7 micrograms of copper) into a Mojonnier extraction flask and add sufficient redistilled water to make a volume of 25 ml. Add 3 drops of cresol red solution and bring the contents of the flask to the yellow color of the indicator range by dropwise addition of C.P. concentrated ammonium hydroxide. Add 2 ml. of the buffer reagent and exactly 20 ml. of the dilute dithizone solution, shake for 10 minutes, and discard the aqueous layer. Add 10 ml. of acidic potassium iodide solution to the flask and shake for 2 minutes. Discard the aqueous layer, and draw off the last 2 or 3 drops with the aid of a pipet bent slightly at the tip. Transfer to a cuvette and determine the transmittance or optical density of the solution in the spectrophotometer at 520 millimicrons, with a blank determination, which has been treated in the same manner as the sample, set at 100% transmittance or 0 optical density. Determine the concentration of copper from a previously prepared calibration curve.

PREPARATION OF CALIBRATION CURVE

Pipette 1-, 2-, 3-, 4-, 5-, and 6-ml. quantities of copper standard (containing 1 microgram of copper per ml.) into six extraction flasks, with a seventh to serve as a blank. Add 5 ml. of 10% sulfuric acid to each of the seven, and add sufficient redistilled water to make a volume of 25 ml. Continue as with the unknown sample and determine the transmittance or optical density in the spectrophotometer at 520 millimicrons. Plot the transmittance, optical density, or log transmittance value against the known concentration of copper in each sample.

EXPERIMENTAL FINDINGS

Recovery of Copper from Solutions Containing Contaminating Metals. Known amounts of copper and other metals whose effects as interfering substances were to be tested were placed in glass-stoppered Mojonnier fat-extraction flasks, 5 ml. of 10% sulfuric acid were added, and the volume was made up to 25 ml. with redistilled water. The procedure described above for copper was then followed. The amount of copper recovered in each case was read from a calibration curve prepared with known amounts of copper.

Table I describes the results obtained upon the addition of metals whose presence is likely in food products or biological material. Overwhelming amounts of lead, zinc, cadmium, cobalt, nickel, and manganese do not influence the quantitative extraction of copper, since these metals do not react with dithizone at the pH chosen. This fact is demonstrated by the quantitative recovery of copper from solutions containing the above-mentioned contaminants when the extraction with acidic potassium iodide is omitted.

Stannous tin and ferrous iron, two possible interfering substances, are of no consequence since the method of sample preparation oxidizes them to their higher valences in which they are not reactive with dithizone.

Although previous workers (*1*, *10*) have said that small amounts of bismuth are extracted at a pH of 2.3 and must be removed by

Table II. Recovery of Copper from Solutions of Varying pH

pH of Solution	pH of Solution after Addition of Buffer	Cu added	Cu Recovered	Error
		Micrograms	*Micrograms*	*Microgram*
1.6	1.8	5.0	5.10	+0.10
1.6	1.8	5.0	4.90	−0.10
1.8	2.0	5.0	4.80	−0.20
1.8	2.0	5.0	4.95	−0.05
2.3	2.4	5.0	4.95	−0.05
2.3	2.4	5.0	4.90	−0.10
6.8	2.8	5.0	4.90	−0.10
6.8	2.8	5.0	5.00	0.0

Table III. Effect of Dithizone Strength upon Recovery of Copper

Dithizone Concentration *Mg./l. CCl_4*	Copper Added *Micrograms*	Copper Recovered *Micrograms*	Error *Microgram*
7.0	5.0	5.05	+0.05
7.0	5.0	4.85	−0.15
7.0	5.0	4.95	−0.05
7.5	5.0	5.0	0.0
7.5	5.0	5.0	0.0
7.5	5.0	5.05	+0.05
8.0	5.0	4.85	−0.15
8.0	5.0	5.05	+0.05
8.0	5.0	5.05	+0.05

potassium iodide extraction, this fact has not been confirmed in the present study. Experimental findings shown in Table I indicate that bismuth is not extracted at this pH.

Silver and mercury, however, are extracted more readily than copper at the pH chosen. For this reason, the method as outlined above will tolerate the presence of only 20 micrograms of either of these two metals. However, since the dithizonates of both of these metals are yellow, their interference is easily detected.

Effect of Small Variations in Extraction pH. Known amounts of copper were placed in Mojonnier fat-extraction flasks, acid and water added to make volumes of 25 ml., and the pH values were varied from 1.6 to 6.8. These pH values were selected because they correspond to the faint pink color on both the acid and alkaline side of the desired yellow color of cresol red indicator. Furthermore they correspond to the maximum error likely to be encountered in pH adjustment during routine analyses.

Table II shows (1) the buffering capacity of the citric acid–phosphate buffer, and (2) that small variations in pH have no effect upon the quantitative extraction of copper when the shaking time is 10 minutes.

Effect of Variations in Shaking Time. The authors' findings in general corroborate those of Bendix and Grabenstetter (*1*) in that in most cases all copper is extracted in 6 minutes. However, because of small individual variations it was decided that a 10-minute shaking time was a safer routine practice.

Effect of Small Variations in Dithizone Strength. Recovery of known amounts of copper was determined in the usual manner, except that the dithizone solution concentration was varied to a slight extent in order to determine its effect upon the transmittance. Clifford (*2*) has pointed out, relative to the determination of lead by the mixed color method, that since the amount of metal present is based upon the measurement of the light absorption of the red phase of the color mixture at a wave length where the absorption of the green dithizone is small, variations in the concentration of free dithizone must be considerable to influence the results appreciably. Table III points out that this statement is just as true in the determination of copper, and that recovery of copper, despite small variations in dithizone strength, is within the limits of error claimed for the method.

Application of Method. Samples of several biological products were analyzed for copper. Recoveries of added copper were made from samples of dried milk, dried eggs, and whole blood. Results of some of the dried milks were checked by the Bendix and Grabenstetter method (*1*). Table IV demonstrates that copper recoveries from biological products by the modified all-dithizone method are satisfactory, and that agreement between this method and the method of Bendix and Grabenstetter is good.

SUMMARY AND CONCLUSIONS

The chemical literature has been studied for the purpose of developing a quantitative, reproducible, rapid method for the determination of copper in biological products. It was decided that the principles embodied in the method of Bendix and Grabenstetter possessed the soundest chemical basis. Experience with the above-mentioned method pointed out that it could be advantageously modified. The authors' innovations consist of a change in the strength of the dithizone solution, the use of the mixed instead of the mono color system for the final determination of copper, and the replacement of separatory funnels with Mojonnier fat-extraction flasks for all extractions. The net effect of these changes is to make the determination more consistently quantitative, more reproducible, and more adaptable for routine use in the hands of relatively inexperienced technicians. Furthermore, by its simplicity the modified method readily lends itself to multiple determinations, a weighty factor in the routine analytical laboratory.

Studies upon the effect of interfering substances show that the method is specific for copper. Interference from overwhelming amounts of other possible metallic ions is shown to be nonexistent, with the exception of silver and mercury, whose dithizonates are easily detectable visually.

Experimental data indicate that pH is not a critical factor if a pH of 2 is approximated, and that a shaking period of 10 minutes allows a fair margin of safety for quantitative extraction of copper from the aqueous solution. Further studies demonstrate that small deviations in dithizone strength have no effect upon the quantitative copper recovery, since the concentration of copper is measured at a wave length where the absorption of the dithizone itself is slight.

Recoveries of added copper from various biological products are excellent, values of better than ±0.2 microgram being obtained.

Table IV. Analyses of Biological Products

Biological Product	Copper Content: Modified dithizone method *P.p.m.*	Copper Content: Bendix and Grabenstetter method *P.p.m.*	Cu Added *P.p.m.*	Cu Recovered *P.p.m.*	Error *P.p.m.*
Dried whole and skim milks	0.98	...	2.0	3.00	+0.02
	0.98	...	2.0	2.80	−0.18
	0.98	...	3.0	3.90	−0.08
	0.98	...	3.0	4.15	+0.17
	0.98	...	4.0	4.80	−0.18
	0.98	...	4.0	5.00	+0.02
	2.39	2.42	...	...	...
	1.34	1.31	...	...	...
	1.39	1.46	...	...	...
	0.59	0.64	...	...	...
	0.58	0.52	...	...	...
	0.99	1.11	...	...	...
	1.34	1.40	...	...	...
	0.45	0.45	...	...	...
Turkey fat	0.30	...	...	...	...
	0.38	...	...	...	...
	0.16	...	...	...	...
	0.64	...	...	...	...
	0.23	...	...	...	...
Dried whole eggs	5.35	...	2.0	7.40	+0.05
	5.35	...	2.0	7.40	+0.05
	5.35	...	4.0	9.30	−0.05
	5.35	...	4.0	9.20	−0.15
Horse blood	0.88	...	1.50	2.50	+0.12
	0.88	...	1.50	2.24	−0.14
	0.80	...	0.50	1.35	+0.05
	0.80	...	0.50	1.23	−0.07
	0.80	...	1.00	1.78	−0.02
	0.80	...	1.00	1.75	−0.05

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NOTES ON ANALYTICAL PROCEDURES

Simple Automatic Control for Vacuum Systems

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A MODIFICATION of a commercial type of Cartesian manostat was so constructed as to bleed air to the system rather than throttle or modulate the vacuum supply. No electrical connections are required and the unit can thus be safely used with systems handling inflammable solvents under conditions where manostats as described by Munch (*1*) might be undesirable.

OPERATION. This modified manostat is illustrated in Figure 1. The valve unit is adjusted so that the rubber disk, *E*, rests on the orifice when the lower end of the wire is approximately 1 mm. from the top of the floating bell. Stopcocks *B* and *H* remain open until the vacuum approaches 10 mm. of the desired reduced pressure. *B* is then closed, while *H* remains partially open. The exact reduced pressure desired is obtained by adjusting the rubber disk on the wire. Functioning of the manostat is indicated by the continuous and rapid axial modulation of the valve unit.

The operation is produced as follows: Since equal pressures must be maintained in the mercury-sealed chamber, *K*, and in chamber *J* leading to the system, a change of pressure in *J* causes an upward or downward displacement of mercury in this chamber, thereby raising or lowering the floating bell, *D*. This in turn opens or closes orifice *G*, thus maintaining the equilibrium in the two chambers.

A 2-mm. orifice controls reduced pressures down to 200 mm. of mercury (absolute) and a 1-mm. orifice controls pressures between 220 and 30 mm. The reduced pressure in a system can be automatically controlled to within ±0.25 mm. of mercury by proper adjustments of the orifice, valve unit, and petcock *B*. After proper adjustments are made the pressure can be controlled for an indefinite period of time with no drifting of pressure within the system.

Two modified manostats as described have been successfully used in the Southern Regional Research Laboratory for several months.

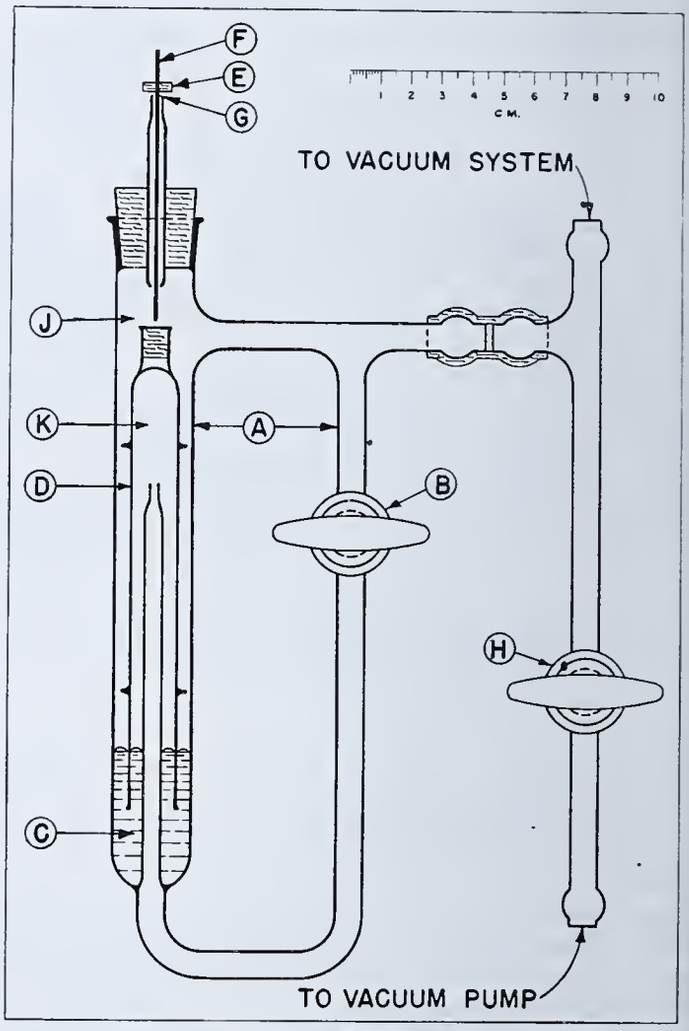

Figure 1. Modified Manostat

A. Main body of manostat
B. Stopcock
C. Section for mercury seal
D. Floating bell
Valve unit consists of adjustable rubber disk, *E*, attached to a rigid noncorrosive wire, *F*, free to move axially through orifice *G*.

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Absorption Spectrum of the Antimony Trichloride–Ergosterol Reaction Product

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DURING the course of investigating the antimony trichloride absorption spectrum of vitamin D at 500 mμ, according to the method described by Nield, Russell, and Zimmerli (*3*), it was observed that solutions of the antimony trichloride reaction products of irradiated ergosterol exhibit an absorption band at 393 mμ as well as at 500 mμ. Further investigation of the absorption spectra of the antimony trichloride reaction products of pure samples of calciferol (vitamin D_2) obtained from Winthrop Chemical Co. and of pure ergosterol obtained from Standard Brands, Inc., indicates the absorption maximum at 393 mμ to be due to ergosterol. The absorption maximum at 393 mμ is sharp and well defined, having a molecular extinction coefficient of 9122 and an $E^{1\%}_{1\ cm.}$ value of 230.0 ± 3% (average of 20 trials). Two additional absorption maxima were observed at 322 mμ and 510 mμ with $E^{1\%}_{1\ cm.}$ values of 68.0 and 25.0, respectively. These measurements were performed on a sample of ergosterol which was shown to be 100% pure by a measurement of its ultraviolet absorption spectrum.

The absorption maximum at 393 mμ was found to be stable for the period from 2 to 10 minutes following preparation of the antimony trichloride–ergosterol reaction product, making it possible to make very satisfactory quantitative measurements of its intensity. The absorption spectrum of the antimony trichloride–calciferol reaction product shows no absorption bands

n the 300 to 400 mμ region. These results indicate that a measrement of the absorption maximum at 393 mμ of the antimony richloride reaction product of irradiated ergosterol should offer a atisfactory method for the quantitative determination of the rgosterol present. The absorption maxima at 322 and 510 mμ are f low intensity and not satisfactory for quantitative measurement, ut are helpful in the qualitative identification of ergosterol. A more detailed report on a spectrophotometric method for the uantitative determination of ergosterol in the presence of itamins D, cholesterol, and 7-dehydrocholesterol based upon the bservation is reported in this issue (*2*).

The spectrophotometric measurements were made with a Beckman Model DU spectrophotometer and incandescent light source. The details of the procedure used in carrying out the antimony trichloride reaction are the same as described by Ewing, Kingsley, Brown, and Emmett (*1*).

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Large-Size Laboratory Soxhlet Extractor

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IN AN investigation involving the extraction of plant lipids, the need arose for an apparatus which could efficiently extract 25 to 30 pounds of finely ground milkweed seeds. Although numerous extractors of different capacities have been described in the chemical literature, it was felt that the type described by Rapp, Woodmansee and McHargue (*1*) was most satisfactory. This extractor can be used for a Soxhlet-type of extraction with hot solvent. In preparing a smaller model of this apparatus, several simplifications in fabrication were introduced, enabling it to be constructed from materials readily available in most research laboratories and manufacturing plants.

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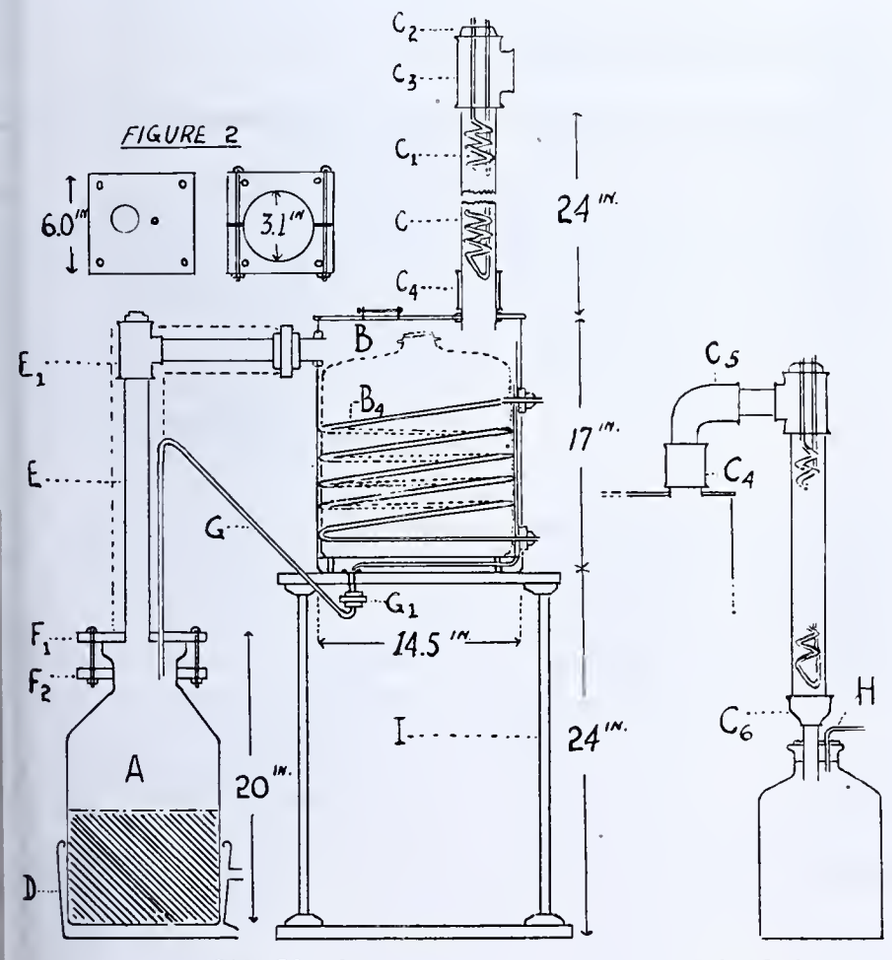

Figures 1, 2, 3, and 4. Diagram of Laboratory Soxhlet Extractor, Showing Details of Construction and Dimensions

CONSTRUCTION OF EXTRACTOR

The extractor is shown in Figure 1. The boiler, *A*, consists of a 5-gallon wide-mouthed Pyrex bottle set in a steam bath, *D*, of appropriate size. The vapor tube, *E*, made of 1.0-inch brass pipe and fittings, with a union adjacent to the extraction chamber, *B*, to facilitate dismantling, is attached to *A* by means of an adapter, *F*. It is advisable to lag the vapor tube, *E*, with 1.0-inch asbestos pipe covering.

The upper part of the adaptor, F_1, consists of a 6-inch square (Figure 2) of 0.25-inch sheet brass having a hole tapped with a 1-inch I.P.S. It consists of a hole for the siphon tube, *G*, and four corner holes for bolts. The bottom half of the adaptor, F_2, is made of 1-inch wood, the inside hole being lined with sheet cork. The details are given in the drawing. A vapor-tight seal is obtained by use of either an asbestos or sheet cork gasket in conjunction with any vegetable-base pipe-joint compound. A sound cork, in conjunction with the pipe-joint compound, may be substituted for the adaptor.

The siphon tube, *G*, is made of 0.31-inch outside diameter copper tubing which is soldered into F_1, and coupled with the extraction chamber, *B*, by means of a 0.125-inch union, G_1. The diameter of *G* for the particular application was found to be important. Tubing of larger diameter was apparently inadequate to provide proper siphon action, the solvent merely spilling over as rapidly as it was condensed. The optimum diameter appears to depend on several operational variables—e.g., rate of distillation, temperature of condensate, etc. A sharp bend is important in facilitating siphoning.

B is made from a sheet-metal batch can of about 12-gallon capacity, the type used in shipping bulk chemicals. It has a beaded top edge, B_1 (Figure 3), and recessed cover, B_2, containing a gasket. The cover and can were coupled in a solvent-tight seal by means of a metal ring (furnished as an integral part of the can), B_3. The best type of gasket was found to be Johns-Manville style No. $322^3/_{16}$-inch packing. After repeated use, it showed no signs of wear.

B is provided with an internal steam coil, B_4, made of 0.31-inch outside diameter copper tubing (about 6 to 7 coils are adequate). This coil serves

a dual purpose, enabling the extraction to be performed at elevated temperatures and facilitating the drying of the extracted material in the final recovery of the solvent. The tubing is attached to B by inserting two 1-inch lengths of 0.5-inch running thread through holes reamed in the side of the can and securing them in place by lock-nuts and fiber washers of appropriate size. The precoiled tubing is placed in the extraction chamber, the ends of the tubing passing through the nipples and being held in place inside the nipples by asbestos tape. Solder is then poured into the remaining space. The openings for the siphon tube on the bottom of the can and for the condenser on the top of the can are made in a similar manner, except that no solder is needed.

A breather tube, to facilitate siphoning, is made of L-shaped copper tubing 0.25 inch in outside diameter, which extends from the siphon tube along the bottom and up the side of the can above the siphoning level.

The material to be extracted is placed in a strong canvas bag with a pull-cord top. The bag fits snugly into the can and is placed on a circular piece of 1.0-inch mesh which is raised slightly from the bottom of the can to facilitate siphoning.

In constructing the stand, I, for the extraction chamber, 0.5-inch pipe is used.

The condenser coil, C_1 is made of 0.25-inch outside diameter copper tubing tightly wound, the ends projecting through a 2.0-inch brass plug, C_2, and soldered in place. C_2 is screwed into a 2.0-inch tee, C_3, which is attached to a 24-inch length of 2.0-inch pipe. The latter is attached to the extraction chamber by means of a 2.0-inch brass coupling, C_4.

To adapt this apparatus for downward distillation, C is dismantled, a 2.0-inch street ell, C_5 (Figure 4), is screwed into C_4, a 2.0-inch nipple is screwed into the street ell, and the condenser is screwed to this nipple as shown. To facilitate collection of the recovered solvent, the pipe size of the condenser is reduced to 0.75 inch by means of a reducing coupling and a 6.0-inch nipple, C_6, which passes through a sound stopper into a wide-mouthed receiver. This stopper also bears an L-shaped piece of tubing, H, as illustrated. If it is desired to dry the extracted material completely and remove all but traces of solvent from the extracted oil, steam may be passed through the coil of the extractor chamber and through the steam bath, D.

A capillary may be inserted through the tee at the top of the vapor tube, E, and gentle suction applied at H for more efficient removal of solvent.

If the material to be extracted is intended for food use and copper and brass may lead to possible future deterioration, block tin tubing may be substituted for copper and Monel fittings used in place of brass.

CONCLUSION

The apparatus described is efficient, giving values that closely approximate quantitative determinations in the conventional all-glass apparatus. The over-all solvent recovery is good, varying from 80 to 90%, dependent on the solvent.

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BOOK REVIEWS

Colorimetry for Chemists. *M. G. Mellon.* 120 pages. The G. Frederick Smith Chemical Co., 867 McKinley Ave., Columbus, Ohio, 1945. Price, heavy board back, $1.00; paper cover, free.

In this 120-page booklet, the author discusses the application of colorimetry in its broader sense to the problems of chemistry. In addition to the chemist's common concept of colorimetry as the measurement of the concentration of a colored constituent, or a constituent which can be transformed into a colored form, the measurement and use of such color quality factors as the dominant wave length, purity, and brightness are described in some detail.

The distinguishing features and limitations of typical commercially available instruments are briefly discussed. Recognition of the limitations of instrumentation has prompted the author to make a very timely plea for more uniformity in the presentation of data for publication, especially for the inclusion of information necessary to the critical evaluation of the data. The author includes a very brief section on the interpretation of transmission and reflectance curves in terms of the corresponding I.C.I. tristimulus coefficients, luminance, dominant wave length, and purity.

This publication should hold considerable interest for chemists planning colorimetric studies; it should aid the experienced in obtaining even more useful and valuable information from available equipment; and it should serve as a guide in the preparation of material for publication in the chemical literature.

O. R. Alexander

Polarographic and Spectrographic Analysis of High Purity Zinc and Zinc Alloys for Die Casting. *A. S. Nickelson* and *J. E. B. Randles.* ix + 117 pages. H. M. Stationery Office, London, 1945. Price, 5 s.

This book, which is composed of four papers, gives a detailed account of the experimental work carried out from 1941 to 1944 by the British Standards Institution Panels in connection with its commission to prepare and recommend methods for the polarographic and spectrographic analysis of zinc and zinc alloys for die casting. The first paper (32 pages) deals with the polarographic determination of impurities. The balance of the text reports on the use of spectrographic methods involving three different types of excitation. The simple condensed spark is employed for the determination of alloying constituents (aluminum, copper, and magnesium). An intermittent alternating current arc of high sensitivity and reproducibility is described for the determination of impurities in samples which are employed as solid electrodes. The concluding paper describes an oxide–direct current arc method which is based on solution of the metal.

The polarographic section disscusses theoretical aspects and pays particular attention to the behavior of tin, copper, and iron. The discussions of the behavior of tin and of the methods for removing interferences, particularly copper, are valuable.

The papers dealing with the spectrographic method describe in detail the aspects familiar to all spectrographers. Factors affecting sensitivity and accuracy, such as types of and variation in excitation conditions, the influence of certain elements on the radiations due to another, the types and shapes of electrodes, the choice of analytical and internal standard lines, etc., have been studied. The last paper also gives an excellent description of plate calibration techniques by two methods, one based on the Schwarzschild law and the other employing a step filter.

The book lacks sufficient literature references, but will prove useful as a practical guide in the analysis of the specific materials to which the methods have been applied. The experiences which are reported when combined with more recent developments should result in modifications which will improve the convenience and accuracies of the applications. Steps in this direction would appear to be (1) in the polarographic method, the incorporation of Lingane's method for removing copper interference by electrolytic separation at controlled potential with a mercury cathode, and (2) in certain spectrographic applications, the use of the high-voltage alternating current arc combined with photometry based on plate calibration and background correction as employed in American practice.

J. Cholak

INDUSTRIAL AND ENGINEERING CHEMISTRY
PUBLISHED BY
THE AMERICAN CHEMICAL SOCIETY
Analytical Edition
WALTER J. MURPHY,
EDITOR

Editorial Policies

NOTHING is certain in the field of chemistry but change. Certainly the field of analytical chemistry has been no exception during the past decade, although perhaps the changes, revolutionary as they are to the analyst, have not been given the spectacular recognition accorded new developments in industrial chemistry.

The editorial policies of the ANALYTICAL EDITION have been determined solely on the basis of meeting promptly and efficiently the changing requirements of the analytical chemist and analytical chemistry. The innovations that should be introduced have been the subject of many earnest deliberations by the editors and the Advisory Board of the ANALYTICAL EDITION. This is particularly true of the past three years, when the board has been active and has met at regular and frequent intervals.

A statement at this time outlining the present aims and purposes of the journal will serve a most useful function, clarifying for our readers the views and policies of the editors. Your editor, Associate Editor L. T. Hallett, and Assistant Editors G. Gladys Gordon and Stella Anderson have cooperated in the preparation of three statements which we hope will be read, digested, and commented on. The first, discussing the scope of the journal, appears on page 218 of this issue; the second, describing style requirements, will be published in the May issue, and this will be followed by a detailed explanation of the reviewing system which will be printed in the June number.

Walter J. Murphy

Scope of the Analytical Edition

WHENEVER a paper is written for publication, the author must decide to which journal it may most suitably be sent. Some authors make a conscious effort to see that the type and style of the article conform to the standards or scope of the journal chosen, as they interpret them; others, it would appear, give little thought to this.

When the ANALYTICAL EDITION was established in 1929, it was intended to serve the analytical chemist by segregating for him worth while contributions in analytical methods and improvements in analytical apparatus in the broad field of industrial and engineering chemistry. While applied analysis has received much attention during succeeding years, the journal has become much broader than its original conception. This, together with the expanded interests of the analytical chemist due to the development of new techniques and instruments, gives point to a short discussion for the guidance of authors on the types of papers which the editors of the ANALYTICAL EDITION consider most suitable, so that the publication may better serve as the journal for analytical chemistry in all its branches.

1. A paper may present an improved or new procedure for the analysis or testing of some element, compound, or property, details of which must be accurately presented, so that others can duplicate the results. The tools used may be chemical or physical, and the procedures may deal with organic, inorganic, physical, or biological chemistry. The physical chemistry, in many cases, may approach pure physics.

2. A review paper may be invited or submitted. Its purpose should be critical evaluation of work in the given field during a specified period, rather than listing references with a brief abstract, for this is more strictly a bibliography. A bibliography is important if it is complete, and can be invaluable as an aid in research, but, in most cases, is necessarily so specialized as to be of interest to only a limited number of investigators and consequently does not often find a place in the ANALYTICAL EDITION. Review papers may deal with either methods or instruments.

3. Papers on the evaluation of analytical results or the statistical treatment or interpretation of analytical and test data are becoming increasingly important. Such methods can point out human and method errors and provide a very useful means of eliminating untrustworthy data or methods. Some papers have been published in this field in the ANALYTICAL EDITION; more are desired.

4. Papers dealing with principles and theory of analytical chemistry are important, in that they form the basis for new approaches to analytical chemistry and the better understanding of old, established methods. They are of interest to all our readers, both academic and practical. Such a paper should be a thoughtful discussion of analysis and does not necessarily include experimental work. Very few papers of this kind have been submitted to the ANALYTICAL EDITION, and some criticism has come from the more theoretically minded analysts that this is a weakness of the publication. We welcome such papers.

5. General papers on the college training of personnel are important, as well as those which deal with the training programs, organization, and operation of analytical laboratories in industry and in research foundations. The function of the research analyst in a well-integrated research program is now receiving greater attention. A successful attack on some research problems is sometimes impossible unless research on methods is worked out in advance, or carried on simultaneously. The analyst on such problems must be of the highest caliber both as a chemist and as an analyst. While these phases of analytical chemistry present problems to many hundreds of people, few papers on these subjects appear.

6. Efficient physical equipment and layout are as important as properly trained personnel. Many times these subjects are discussed by correspondence between the heads of analytical laboratories, but papers discussing these problems would serve a larger audience. Floor plans of analytical laboratories have been published in the ANALYTICAL EDITION from time to time, but a critical statement of the reasons for the selection of a particular laboratory setup would enhance their value.

7. The application and development of instruments designed for analyses are likely to receive greater attention from authors. The increase in the importance of this field as envisioned by your editors is indicated by the monthly articles on instrumentation, written by Ralph H. Müller, that began to appear in January of this year.

There may be other subjects which our readers would like to see discussed in the ANALYTICAL EDITION. We welcome your suggestions.

Optical Methods in Electrophoresis

Principles, Apparatus, Determination of Apparatus Constants, and Application to Refractive Index Measurements

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The optical equipment developed at The Rockefeller Institute for the quantitative study of refractive index gradients in solution is described, together with suggestions for its installation and adjustment. This equipment, which is based on the Foucault-Toepler schlieren method, also incorporates the author's scanning modification and the cylindrical lens arrangement of Philpot and Svensson. Although the chief use of the apparatus is to record the moving boundary patterns in the electrophoretic analysis of proteins by the Tiselius method, this application has been adequately discussed elsewhere and is not included here. The precise measurement of refractive index differences in aqueous solutions represents, however, a recent application of the apparatus and is described.

THE optical equipment, as developed at The Rockefeller Institute, for use in the electrophoretic analysis of proteins by the Tiselius moving boundary method (*19*), has been duplicated in other laboratories and has been made available commercially. (The complete apparatus, in essentially the form described here, is supplied by the Klett Mfg. Co., New York, N. Y.) As a result, over forty installations are now in use both here and abroad. Although based on Tiselius' adaptation of the Foucault-Toepler schlieren method, the details of the apparatus have been evolved in this laboratory and an adequate description of them, which is given in this paper for the first time, should prove of value to investigators in the field of electrophoresis. With these same workers in mind a description of precise methods for testing and focusing the equipment and for determining the constants of the apparatus is also included. Not included in this article are descriptions of the electrophoresis process nor of the moving boundary cell and its accessories, since these have been adequately treated elsewhere (*7*, *9*, *10*).

Precise interpretation of the patterns that are obtained in the electrophoresis and the diffusion of proteins has been hindered, however, by the lack of adequate data on the refractive indices of solutions of these materials. This is especially true at 0°, the temperature at which proteins are frequently studied. Since the optical equipment described here is well adapted for such measurements on aqueous solutions in general, this application is discussed.

PRINCIPLE OF THE SCHLIEREN METHOD

The essential features of the schlieren method may be described with the aid of Figure 1.

An image at P of the point source of light, S, is formed by a lens, L. The objective, O, of a camera is placed just to the right of P and is focused on L, thereby forming an image of this lens on the screen at G.

Each portion of L is capable, of course, of forming an image of point S and if this lens is perfect all the images thus formed will superimpose at P. If, however, a portion of the lens is imperfect, the image formed by this area will not coincide with the other images.

In Figure 1 an exaggerated imperfection, idealized as a tiny prism, is indicated at A and the image formed by this portion of the lens as being at Q. Normally this imperfection is not visible in the image of the lens at G, since it is a property of the camera objective that all rays originating in its object plane are brought to focus at the conjugate points in the image plane regardless of the portion of the objective through which they pass. It is assumed, of course, that the intervening regions are optically homogeneous. If, however, the opaque screen, D, is raised so as to intercept the light forming image Q, while allowing that forming image P to pass, the region of imperfection will become visible at A' as a dark area, or shadow, in the otherwise uniformly illuminated image of the lens, L. This will be recognized as a simple example of Foucault's knife-edge test.

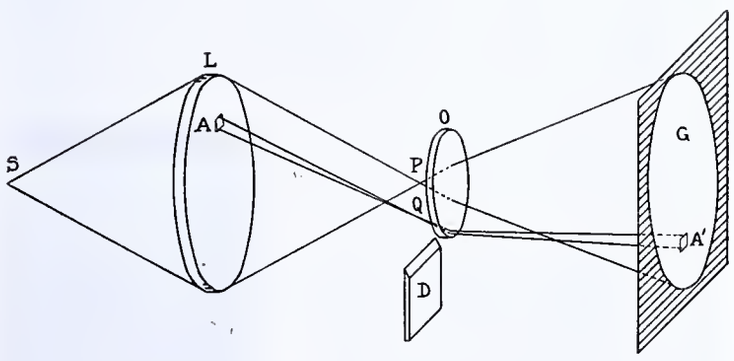


Figure 1. Foucault-Toepler Schlieren Method

Toepler observed that if the lens, L, were free from imperfections the camera could be focused on a plane in front of that lens and variations of refractive index in, or near, that plane could be made visible as shadows, just as the lens imperfections were rendered visible in Foucault's test. The shadows he called schlieren and the method the schlieren method. The lens, L, of Figure 1, is called the schlieren lens, the intercepting screen, D, the schlieren diaphragm, and the camera the schlieren camera. An excellent discussion and bibliography of the schlieren method are given in Schardin's monograph (*13*).

The form that the schlieren method takes for the observation and photography of refractive index gradients that occur in moving boundary and diffusion studies is shown diagrammatically in Figure 2. In this case gravity ensures that the density and, in general, the refractive index are uniform throughout each horizontal layer in the boundary.

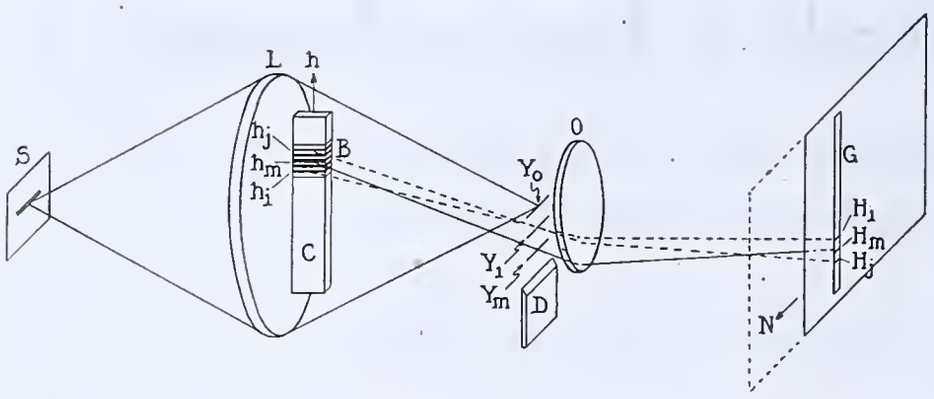


Figure 2. Schlieren Method for Study of Refractive Index Gradients in Solution

The schlieren lens, L, then forms at Y_0 in the plane of the schlieren diaphragm, D, an image of the illuminated, horizontal slit, S. The camera lens, O, is now focused on the cell, C (Figure 2), and forms an image of it on the screen at G. If the fluid in the cell is homogeneous this image will be uniformly illuminated. Suppose, however, that a boundary between, for example, a protein-bearing solution and a buffer solution is present in the channel. Such a boundary will consist of a region, B, of finite thickness, in which the concentration and hence the refractive index, n, vary continuously with the height, h, from its constant value in one homogeneous solution to that in the other solution. The gradient, dn/dh, of refractive index, which may be abbreviated to n', will thus vary from zero to a maximum, and back to zero.

In Figure 2 the variation of the gradient in the boundary is indicated by the density of the shading. Each layer of solution in this boundary acts, therefore, like a prism and deflects the light passing through it. If the denser solution underneath has the higher refractive index, as is usually the case, the deflection is downward. Moreover, if a layer is sufficiently thin the gradient may be considered constant in it and the light passing through this layer forms an image of the slit below the normal one at Y_0. Two such displaced slit images are indicated in Figure 2. The one at Y_m is formed by the layer of solution at h_m having the maximum value of dn/dh—i.e., n_m'. That at Y_1 is the superposition of the two images formed by the two layers at h_i and h_j for which n' has identical values. Actually, as is shown in Figure 3 for a typical case, the displaced slit images formed by all the layers in the boundary combine to give, as first described by Gouy (*4*), an interesting pattern of interference in the region from Y_0 to Y_m.

If, now, the upper edge of the schlieren diaphragm, D (Figure 2) is raised to level Y_m the light through the layer at h_m in the boundary will be intercepted and a dark, narrow band will appear at the conjugate level, H_m, in the image of the channel. On raising this diaphragm further—e.g., to level Y_1—the light from h_i and h_j, together with that from all intervening layers in the boundary, will be intercepted and the dark band in the image of the channel will broaden until the edges of the band are at the conjugate levels H_i and H_j. This, obviously, can be continued until the undeflected rays at Y_0 have been intercepted and the entire image becomes dark.

With a single boundary in the channel a series of fourteen photographs was taken, with the schlieren diaphragm raised a fixed distance between each exposure, and is shown in Figure 4, *a*. The progressive broadening of the schlieren band is apparent. If an exposure is made when the displacement of the diaphragm from the normal slit image is $Y_1 - Y_0$, for example, the edges of the band in that photograph are then conjugate to the levels in the channel at which the refractive index gradient has a value proportional to $Y_1 - Y_0$. The proportionality factor is (*21*), to a very close approximation, the product $a \times b$, in which a is the dimension of the channel parallel to the optic axis and b is the optical distance from the center of the channel to the schlieren diaphragm

$$Y - Y_0 = \Delta Y = abn' \qquad (1)$$

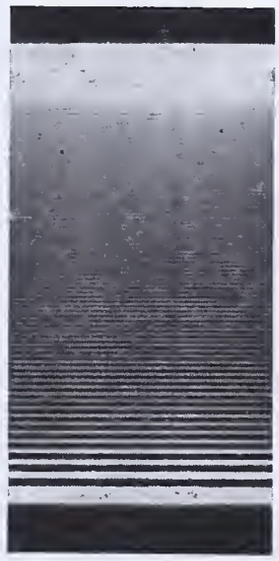


Figure 3. Interference Patterns at Schlieren Diaphragm

SCHLIEREN SCANNING METHOD

In the schlieren scanning procedure (*8*) the stepwise recording shown in Figure 4, *a*, is made continuous.

The image of the cell at G (Figure 2) is masked by a vertical slit of 0.2-mm. width and a photographic plate is moved in the direction, N, of the arrow at a constant rate across this slit. Actuated by the same mechanism, diaphragm D is given a steady movement upward. The resulting photographic positive for the boundary of Figure 4, *a*, is shown in Figure 4, *b*, and is called the boundary pattern. It will be noted that the schlieren bands of Figure 4, *a*, correspond to narrow vertical sections in the complete pattern. For purposes of publication it is the almost universal practice to turn the pattern through 90° as shown in Figure 4, *c*. If more than one boundary is present in the channel each appears as a separate peak in the complete pattern.

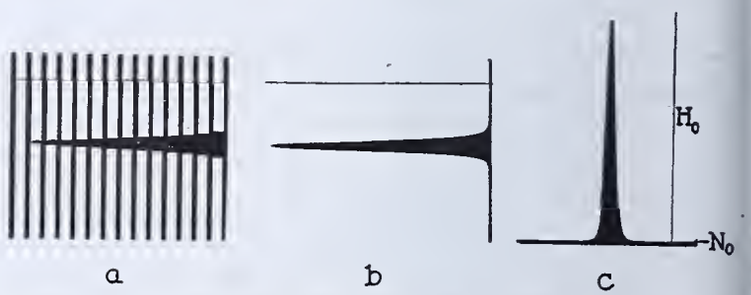


Figure 4. Schlieren Band and Scanning Photographs of a Single Boundary

The outline of the shaded area in Figure 4, *c*, is, therefore, a plot of the refractive index gradient in a thin layer of solution in the channel as ordinate against the height of the layer as abscissa. If E is the enlargement factor for the camera, the scale of abscissas for this plot is Eh, which will be designated as H. The ordinate scale is $abMn'$, designated as N, in which M is the ratio of the rates at which the plate and diaphragm move in the scanning process. The vertical line at H_0 in Figure 4, *c*, is due to a graduation on the cell and is taken as the origin of abscissas. The origin of ordinates is the horizontal line at N_0 joining those portions of the pattern outline that correspond to layers in the channel in which the solution is homogeneous. This is the "base line" of the pattern. In the ideal case this line is straight and horizontal. In practice, however, it is distorted slightly by imperfections in the lenses and in the cell and thermostat windows. Allowance for these distortions is made with the aid of a scanning photograph of the cell when it contains a homogeneous solution.

CYLINDRICAL LENS METHOD

In the scanning method described above the complete pattern is not visible in the focus of the camera but only on the developed photographic plate. It is convenient, however, to be able to observe the pattern directly on the ground glass. The cylindrical lens method (*11*, *16*, *17*) makes this possible and represents a useful addition to the schlieren camera.

In this method the schlieren diaphragm takes the form of a narrow slit, I (Figure 5), that is inclined to the vertical at an angle of, say, 45°. Moreover, a cylindrical lens, with its axis vertical, is interposed at C and is focused on the inclined slit and on the screen at G. Otherwise, the arrangement is as shown in

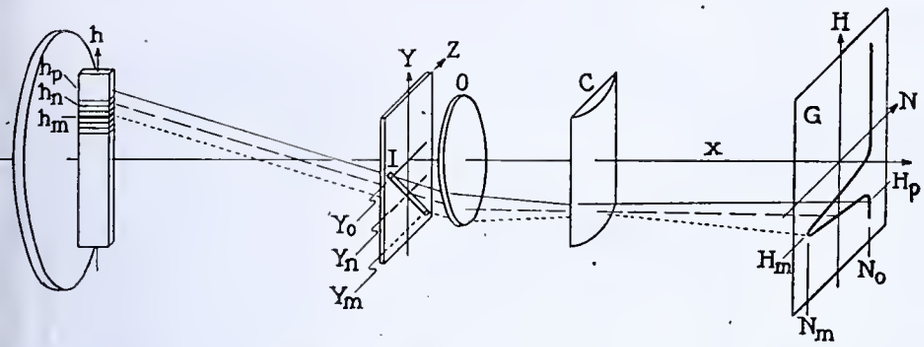


Figure 5. Cylindrical Lens Method

Figure 2. The presence of the cylindrical lens does not alter the vertical coordinate, H, of a point in the image of the cell but, in conjunction with the inclined slit, may displace it along the cross axis, N. As is shown below, this lateral displacement is proportional to the deflection that is suffered by the given pencil of light as it passes through the cell.

As before, if the fluid in the channel is homogeneous, all the light passing through it is concentrated in the normal slit image at Y_0. As can be seen in Figure 5, only the light forming the extreme left-hand portion of this image passes the inclined slit. As the figure is drawn these rays lie to the left of the optic axis, x, and are deflected, therefore, to the right of that axis by the cylindrical lens, thus forming a narrow vertical band of light on the screen at N_0. This is the base line of the pattern. One undeviated pencil of light, that through the layer of homogeneous solution at Y_p, is traced in Figure 5 as a full line and forms one element of the base line at the conjugate level, H_p, in the image plane.

With a boundary in the channel, however, the light through the layer having the maximum gradient is deflected to Y_m. As is indicated by the dotted line in Figure 5, only the rays forming the right-hand portion of this image pass the inclined slit. The camera objective, O, brings these to focus at the same level, H_m, as in the absence of the cylindrical lens, but these rays pass the inclined slit to the right of the axis. Consequently they are shifted to the left by the cylindrical lens and form the peak of the pattern at N_m.

The path of the light through another portion of the boundary is indicated by the dashed line in Figure 5 and forms the corresponding element in the pattern. All other elements in the complete pattern are formed similarly. The focusing action of O on the channel ensures that the vertical coordinate, H, of the pattern corresponds to height h while the focusing action of the cylindrical lens, C, on the inclined slit requires that the N coordinate depend upon the lateral position at which the light passes this slit. As in the scanning procedure the factor of proportionality between h and H is the camera enlargement, E. The dependence of the N coordinate upon the refractive index gradient in the cell may be obtained as follows:

As is evident in Figure 5, the displacement, $N - N_0$, of a pencil of light that has been deflected in the boundary depends upon the angle, θ, that the inclined slit makes with the vertical and the lateral magnification, K, of the cylindrical lens. If Y_0 and Z_0 are the vertical and cross-axis coordinates, respectively, of the point of intersection of the inclined slit and the normal slit image, then the equation reads

$$N - N_0 = K(Z - Z_0) = K(Y - Y_0) \tan\theta = Kabn' \tan\theta \quad (2)$$

A pattern obtained with the aid of the cylindrical lens method is shown in Figure 14. Although a slit as diaphragm has been described here in conjunction with the cylindrical lens, one may also use a diagonal straightedge (*11*) or a diagonal bar (*12*). Moreover, it is also possible to use either a slit (*14*), a bar, or a straightedge, in this case in a horizontal position, with the scanning procedure. With either method the straightedge gives the pattern as the outline between a dark and a light field, the slit gives a more or less narrow band of illumination on a dark background, and the bar gives a dark band on a light background. No comparative study of the three types of diaphragm has been made, however. For visual observation of the pattern with the aid of the cylindrical lens method the author prefers a slit. In the permanent photographic record of an experiment it is essential, however, to have a reference line of known orientation, such as that at H_0 in Figure 4, *c*, and this is difficult to obtain with a slit as diaphragm. Consequently a straightedge as diaphragm is used for this purpose, together with the scanning procedure, thereby avoiding the errors accompanying the use of an uncorrected cylindrical lens.

OPTICAL EQUIPMENT

In the diagrams used thus far to illustrate the principles of the schlieren method, no attempt has been made to preserve the proper scale nor to indicate the construction of the various elements of the optical system. Moreover, the constant-temperature water bath in which the cell is immersed has been omitted from Figures 2 and 5. A scale diagram of the complete apparatus, as developed at The Rockefeller Institute, is shown, therefore, in Figure 6. In this modified isometric drawing the dimensions along the cross axis are on a scale one half of that used for the horizontal and vertical distances. In so far as is practicable the letters on this and subsequent diagrams identify the same optical element as in Figures 1, 2, and 5. Proceeding from left to right in Figure 6, for example, S indicates the illuminated horizontal slit, L the schlieren lens, D the schlieren diaphragm, and G the ground glass or photographic plate.

It may be apparent from Figure 6 that the lenses are of rather long focal length. Together with the other elements of the system they must be rigidly mounted on a firm support and provided with sufficient adjustments for their proper alignment. This is the purpose of the optical bench.

The Optical Bench. The optical bench consists of two 5.94-meter (19.5-foot) steel channels, C (Figure 6), bolted together by means of the plates, H. The bench is supported on two concrete blocks, B, and passes through a trough, R, in the block, B', that carries the thermostat, T. In order to minimize vibration the blocks rest on small shock-absorbing Keldur mats placed, at the corners of each block, on a firm foundation, preferably a ground floor. A sensitive spirit level is used in leveling the bench.

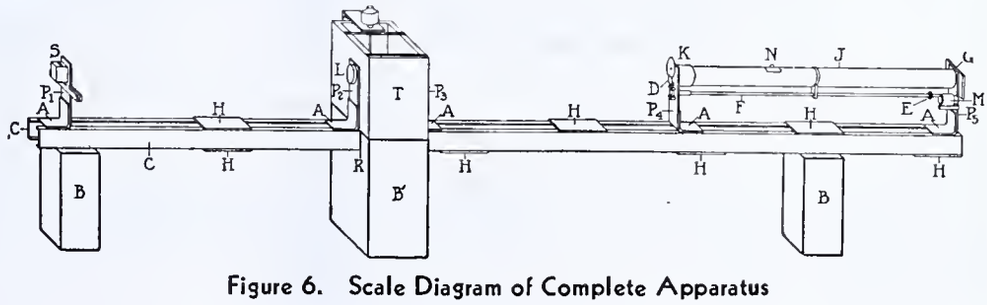


Figure 6. Scale Diagram of Complete Apparatus

Each component of the optical system is carried by a steel plate, P, that is bolted through slotted holes to the vertical faces of the steel angles, A. As shown in more detail in the lower part of Figure 7, the horizontal face of an angle, A, is clamped to the flanges of the channels, C, with the aid of a plate, B. Adjustment along all three axes may thus be made in the alignment and focusing of the components. Once the adjustments have been made it is essential that they be not disturbed by the routine use of the apparatus. First consideration is given, therefore, to the sturdiness of the equipment rather than ease of adjustment.

The Horizontal Slit. The illuminated horizontal slit, S (Figure 6), that serves as the source of light is also shown in detail in Figure 7. This is a view in perspective with the lamp and lamp house removed. The horizontal dimension of the slit shown here is 25 mm., which is about the length of the arc in the 100-watt G.E. mercury lamp, Type H4, used to illuminate the slit. The vertical dimension of the slit is adjustable, both of the leaves, L_1 and L_2, being movable in a T-slot cut across the face of the plate, Q. Since no condensing lens is used, the lamp is placed as close to the slit as its glass bulb permits. Plate Q is mounted therefore, on the steel plate, P_1', but separated from it so that the

socket does not limit the proximity of the lamp to the slit. Both Q and P_1' have openings to pass the light from the slit to the schlieren lens.

Although hidden by P_1' in Figure 7, a holder for 5-cm. (2-inch) square filters is placed in front of the opening in that plate. Either glass or gelatin film filters may be used to isolate the desired lines of the mercury spectrum. A table of the available filters for this purpose is given by Strong (*15*). The author has found a Wratten filter No. 22 (yellow) mounted in C glass satisfactory for work with clear, colorless solutions. If, however, one of the solutions meeting at a boundary is colored, a solution of hemoglobin, for example, it is generally necessary to use a filter with an absorption similar to that of the colored material in order to reduce the shading, at the boundary level, of the illuminated portion of the pattern. A similar shading also occurs in the image at the boundary between a clear and an opalescent solution. In this case the shading is due to the scattering of light by suspended particles and can be reduced, as was shown by Treffers and Moore (*20*), by using red or infrared light.

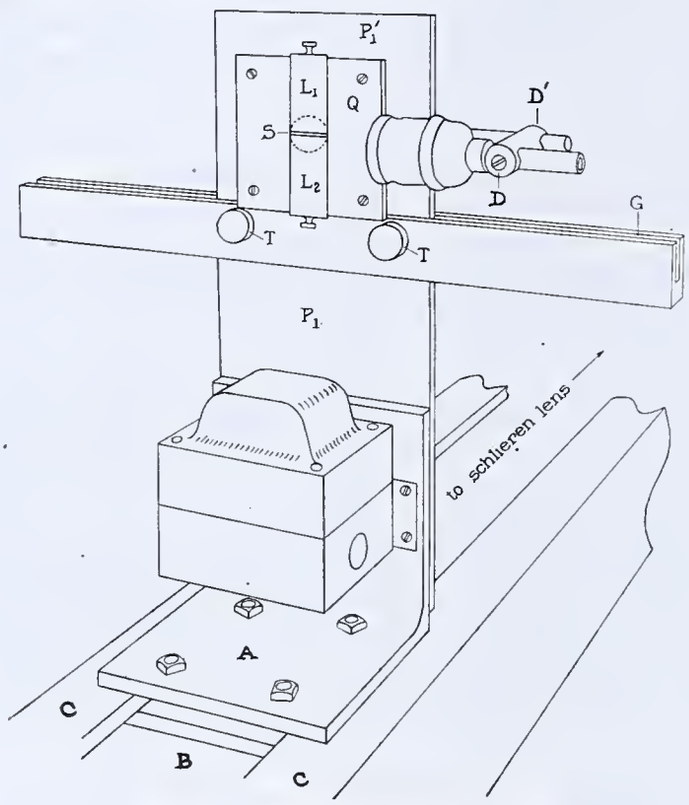


Figure 7. Illuminated Horizontal Slit

Since the mercury lines in the red are of low intensity, a tungsten lamp or Nernst glower (Stupakoff Laboratories, Latrobe, Pa.) may be used for the longer wave lengths. Moreover, it is convenient to have the two sources interchangeable and prefocused. A second slit, illuminated by an appropriate source, is mounted, therefore, on a plate similar to P_1', but not shown in Figure 7. By loosening the thumbscrews, T, plate P_1', carrying the mercury lamp, may be moved out of position along the groove, G, and be replaced by the plate bearing another source of light.

THE SCHLIEREN LENS. The schlieren lens, L (Figures 2 and 5), is undoubtedly the most important single element in the optical system. Upon the performance of this lens depends, in a large measure, the quality of the patterns that one obtains.

In the equipment described here the schlieren lens is an air-spaced achromat with corrections similar to those of a telescope objective, except that it is designed for use at unit magnification and allowance is made for the 30-cm. (12-inch) thickness of water in its image space.

The author's lens has a focal length of 937 mm. and a diameter, d, of 100 mm. Since this diameter is but slightly greater than the height of the channel in the cell, it is essential that this lens be uniformly illuminated. This imposes certain restrictions on the dimensions of the light source and slit that may be derived with the aid of Figure 8.

Let J be the vertical dimension of the source at I (Figure 8) over which the light intensity is constant and w the corresponding dimension of the slit at S. Since the area of the light source exposed by the slit at a given level in the plane of the lens at L is constant for all points between C and D, the illumination between these levels is uniform. In the penumbra above C and below D the light intensity decreases. If the center of the slit is taken as the origin, the coordinates at A are $-x_I$, $-J/2$ while those of B are 0, $-w/2$. The equation of line ABC is therefore $w = (Jx - 2hx_I)/(x_I + x)$. If the schlieren lens is to be uniformly illuminated the ordinate for C at $x = x_L$ must be $d/2$ or greater. The maximum permissible slit width, w_m, is, therefore, $w_m = (Jx_L - dx_I)/(x_I + x_L)$.

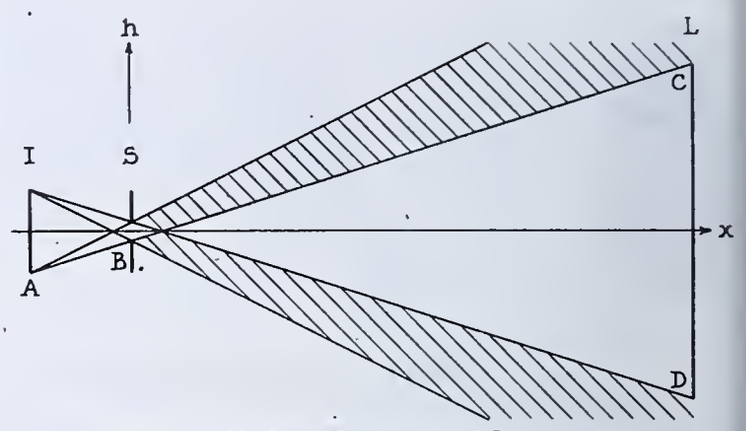


Figure 8. Uniform Illumination of Schlieren Lens

Since the bulb of the mercury lamp has a diameter of 32 mm., x_I = 16 mm. The value of J for the arc is about 1.5 mm. Since d = 100 mm. and x_L = 1850 mm., w_m is about 0.6 mm. Fortunately, this is greater than the optimum slit width of about 0.2 mm. and therefore affords a reserve of illumination for those occasions when the absorption or scattering by a solution is such that the other available exposure controls are inadequate. It is clear, however, that the light source must be accurately aligned with the slit and schlieren lens. This adjustment can be made after loosening the setscrews at D and D' (Figure 7).

Owing to the small horizontal dimension of a channel, 3 mm., no difficulty is experienced in achieving uniform illumination in this direction.

In order to exclude light from the camera that does not pass the channel of the cell the schlieren lens is provided with a mask as shown at M (Figure 9). Moreover, the actual cell consists of a U-shaped channel, in either side of which boundaries may be present. In the case of the cylindrical lens method the patterns formed by the boundaries in the two sides overlap. When using this method it is necessary, therefore, to mask one side when viewing the other. This is done by rotating the plate, A, on the pivots, B and B', to cover one of the slots. The mask swings on the hinges, H and H', to expose the entire lens.

THE LOW-TEMPERATURE THERMOSTAT. In order to control the temperature of the solutions in the cell it is immersed in a constant-temperature water bath, T (Figure 6). Since most work with proteins is done at a temperature of 0.5° it is essential that condensation of moisture on the thermostat windows be minimized. The construction shown in cross section in Figure 10 is effective except when the dew point approaches the room temperature.

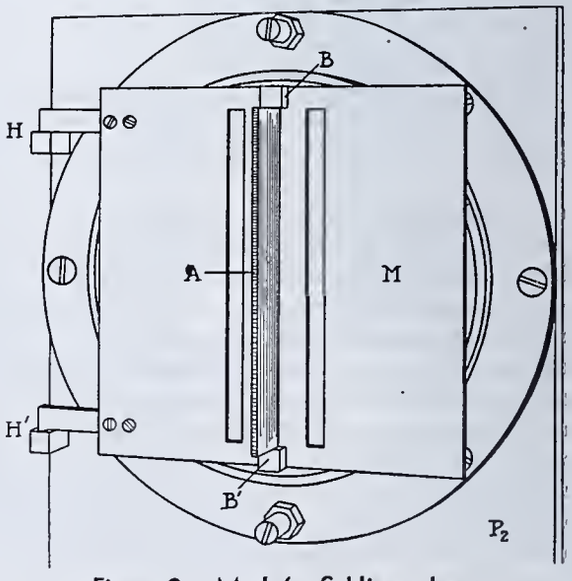


Figure 9. Mask for Schlieren Lens

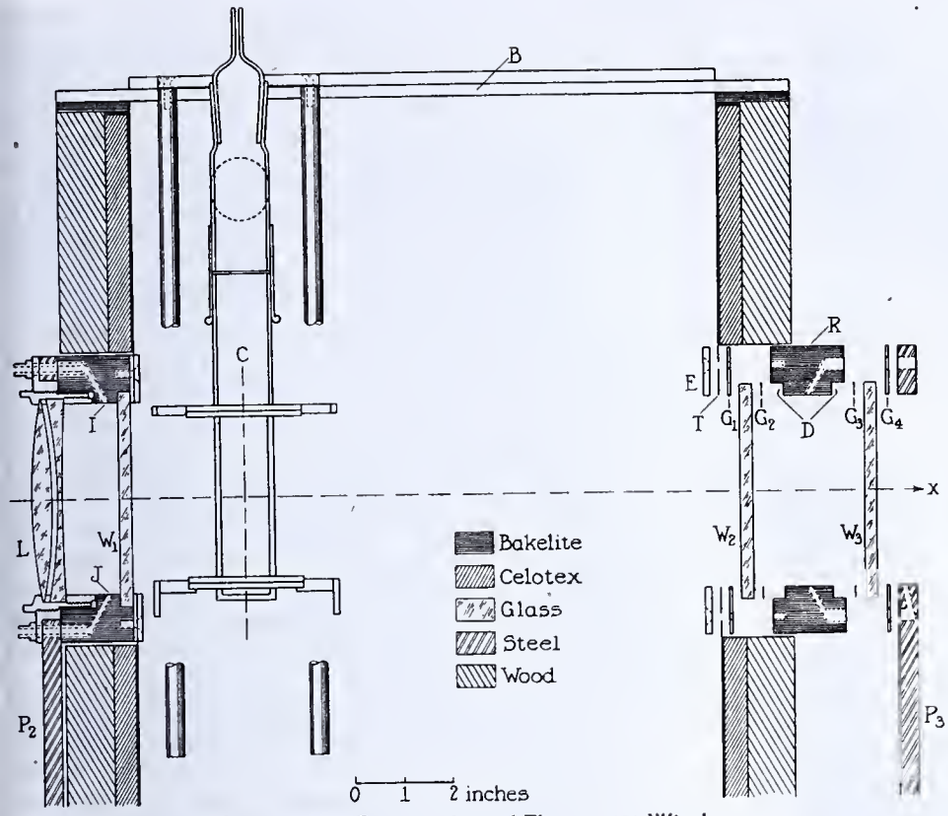


Figure 10. Construction of Thermostat Windows

In this figure the right-hand window is shown unassembled. The glass disks, W_2 and W_3, are seated against the Pliofilm gaskets, G_2 and G_3, in the recesses, D, of the Bakelite ring, R. The rubber gasket, G_1, and the tinned-copper wall of the tank, T, are then clamped between R and the brass ring, E, with the aid of machine screws threaded into the Bakelite ring, thus making the tank watertight at this window. Similarly the space between W_2 and W_3 is made airtight by clamping W_3 and gasket G_4 between R and the steel plate, P_3.

The left-hand window is similar to that on the right except that the schlieren lens, L, replaces one of the glass disks. Provision is made, at I and J for example, for the passage of dry air through the space between the elements of each window.

The Schlieren Scanning Camera. As in the case of an ordinary photographic camera, the schlieren scanning camera is provided with an objective, a means of varying its aperture, a shutter, a bellows, and a plate holder. In addition, provision must be made for a uniform vertical movement of the schlieren diaphragm that is synchronized with the horizontal movement of the photographic plate. Obviously this synchronization must be virtually complete if the ratio, M, of the plate movement to that of the diaphragm is to be a true constant. The components of the camera are described below in the order in which they appear, from left to right, in Figure 6.

The straightedge, D (Figure 6), that serves as schlieren diaphragm in the scanning procedure is shown in more detail at D in Figure 11. It is carried by the rods, R, that slide in the tubes, T. In order to couple the movement of this diaphragm with that of the photographic plate a threaded rod, A, is also attached to D and passes through a threaded bushing in the gear, G. This is driven by the gear, G', attached to the end of the shaft, F, that runs the length of the camera.

Also shown in Figure 11 is the disk, B, that is provided with a series of openings. By rotation of the disk any one of these openings may be placed in front of the objective mounted at O in plate P_4. It fulfills the same function as the iris diaphragm of an ordinary camera. The shapes of the openings are such, however, that they provide a control of the exposure without restricting the lens aperture vertically, thus ensuring that an opening will not act as a schlieren diaphragm.

On the same axis with disk B, but rotating independently of it, is a second disk, C, that is provided, in one quadrant, with a circular hole matching the lens aperture and, in the opposite quadrant, with an adjustable, sector-shaped opening. Driven through spur gears by a small synchronous clock motor, not visible in the figure, this disk serves as the camera shutter. When viewing the pattern on the screen, or photographing it with the scanning device, this shutter is not used, the circular hole in it being left directly in front of the lens. In the photography of schlieren bands and cylindrical lens patterns the sector opening is adjusted to give the proper exposure. (This adjustment is made after loosening, with the aid of a small spanner wrench, the threaded collar that clamps the two leaves of the sector.)

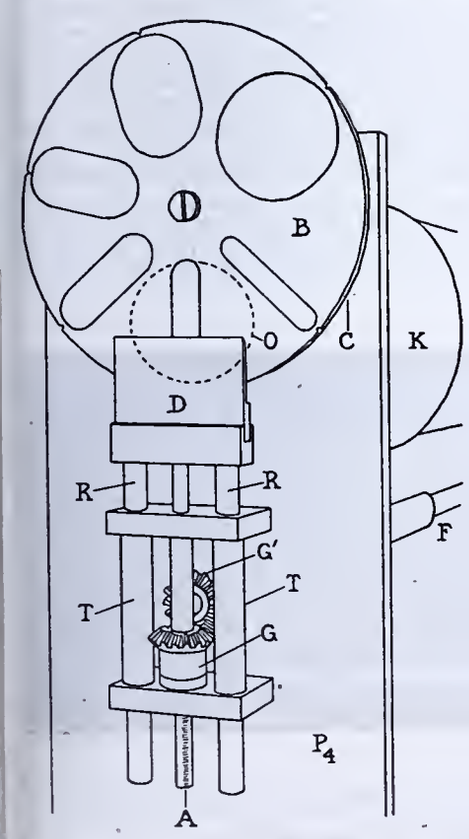


Figure 11. Schlieren Diaphragm

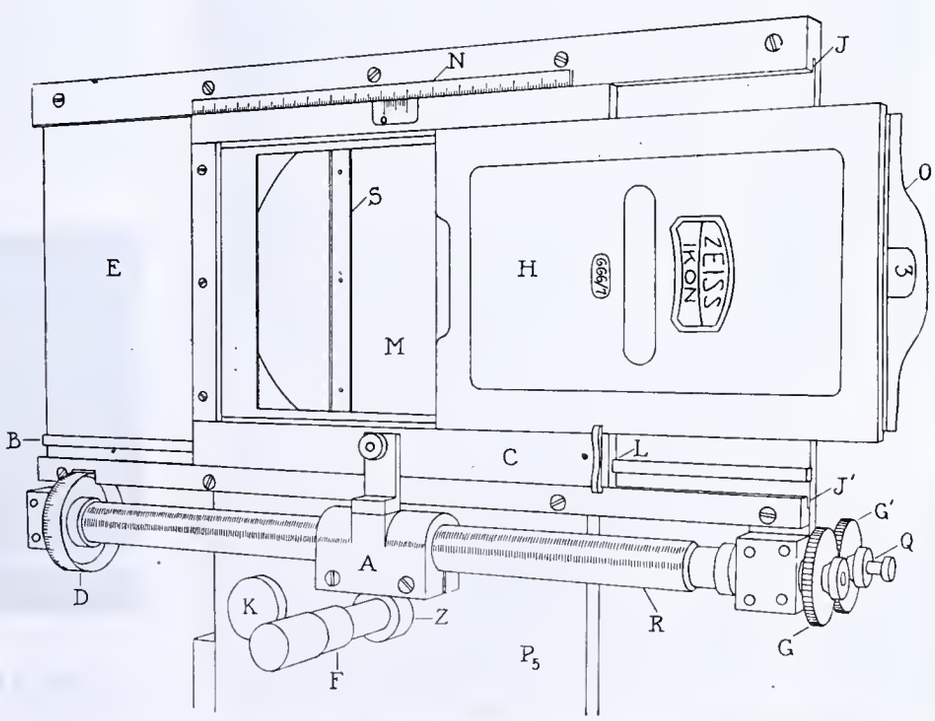


Figure 12. Plate Carriage

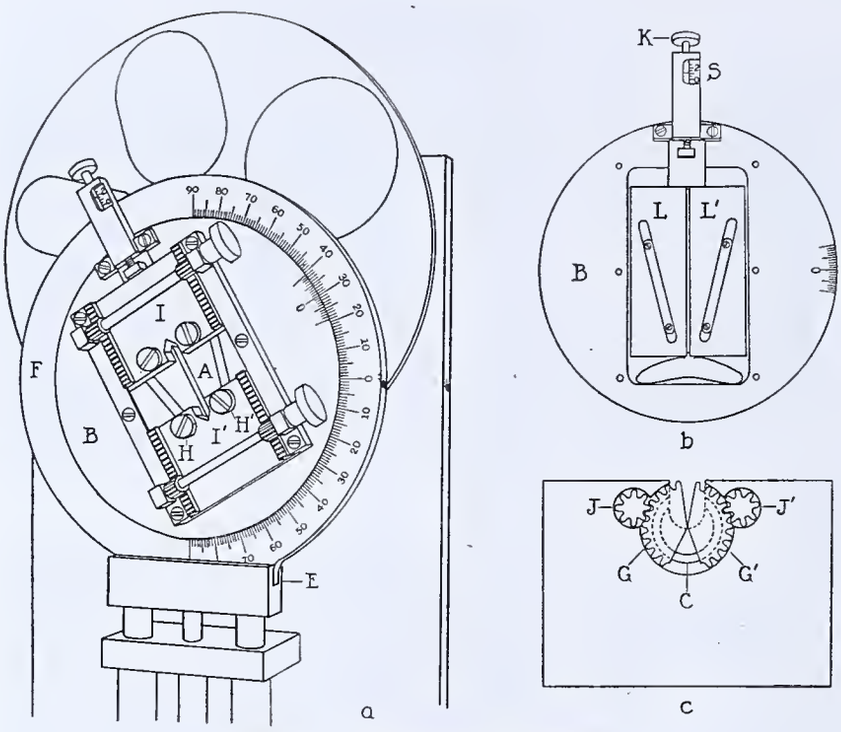


Figure 13. Inclined Slit Diaphragm

The shutter motor is started, and the dark slide of the plate holder removed as an opaque quadrant of the shutter disk moves across the lens. The quadrant containing the open sector then crosses the face of the lens and makes the exposure, after which the dark slide is reinserted as the second opaque quadrant cuts off the light. On completion of the revolution the circular opening is again in front of the lens.

The camera objective, O, is a 50-mm. achromat with the same focal length, f, and corrections as the schlieren lens. Since it is used at unit magnification the length of the aluminum tube, J (Figure 6), that serves as a "bellows" is approximately $2f$. Since it is not practicable to specify exactly this focal length, some adjustment in the length of the camera is essential. This is provided by the collar K (Figure 11), into which one end of the tube slides.

The arrangement for moving the photographic plate horizontally, as the schlieren diaphragm is raised vertically, is shown in Figure 12. The holder, H, for 9 × 12 cm. plates is shown partially inserted into the plate carriage, C. Although this carriage is provided with flanges that move in slots, J and J', these function merely as light baffles, the clearance being sufficient to ensure that the horizontal alignment is maintained solely by the bar, B, that fits into the carefully lapped groove at L. The carriage is loosely coupled, by means of a thumbscrew, to the bushing, A, and driven by the threaded rod, R. This rod is then connected, through gears G and G', to a shaft that is parallel to R but hidden by it and plate P_5 except for the end at Q. This shaft engages, through a clutch operated by the knob, K, the slow-speed shaft of the synchronous motor and reducing unit indicated at M in Figure 6.

Also hidden by the plate, P_5 (Figure 12), are the spiral gears that connect shaft Q with the rod, F (Figure 6), that drives the schlieren diaphragm. Since the spiral gear ratio is fixed at 3 to 1 and the threaded rods, R (Figure 12) and A (Figure 11), have the same pitch of 1 mm., the ratio of the plate travel to that of the diaphragm depends only on the gears at G and G' (Figure 12). If these gears are similar, as shown in the figure, the lateral magnification, M, is then 3. Other pairs of gears can, however, be used to obtain other magnifications and this adjustment in the scanning procedure will be recognized as equivalent to changing the angle of the diaphragm slit in the cylindrical lens method.

The mask for the cell image in the scanning procedure consists of a disk, M (Figure 12), that is provided with an adjustable slit, S. (The width of both this slit and the horizontal one at the light source is adjusted with the aid of a "feeler" gage.) The mask fits into a recess in plate E, so that its surface is flush with that of the plate. Removal of the mask, with the aid of a thumbscrew inserted into a hole provided for the purpose, permits viewing the image on a ground glass that may be substituted for the plate holder, H. In the position shown in Figure 12 the slit exposes the central portion of the image of one side of the channel of the cell. The other side of the channel is exposed by turning the mask through 180°.

In order to determine the position of the schlieren diaphragm while viewing the ground glass, a scale is provided at F (Figure 12). Actually this is a micrometer head with the shank clamped in the collar, Z, while the spindle extends through the plate, P_5, and is keyed into a slotted hole in the end of the rod, F (Figure 6). Although the scale is graduated in hundredths of a millimeter, the readings must be doubled, since the pitch of the micrometer thread is one half that of the rod driving the diaphragm. If this scale is set to read zero when the camera objective is just covered by the diaphragm, that lens will be completely exposed at a reading of 25 mm.

The lateral position of the photographic plate is given to the nearest 0.1 mm. by the scale and vernier at N, or to 0.01 mm. on the graduated drum, D.

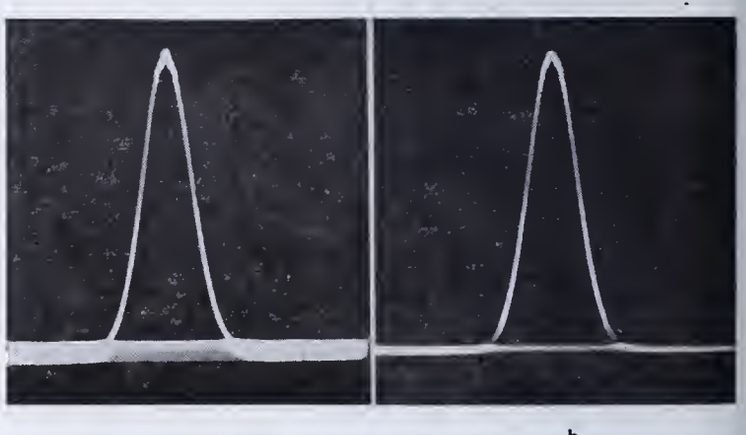
Modification of Camera for Use with Cylindrical Lens Method. With minor modifications of the camera described above it is possible to use the cylindrical lens method interchangeably with the scanning procedure. The modifications consist in the substitution of an inclined slit for the horizontal straightedge as schlieren diaphragm and the insertion of a cylindrical lens between the camera objective and the photographic plate.

The possibility of using the scanning and cylindrical lens procedures interchangeably has certain advantages. Thus it is convenient to be able to view, as in the Philpot-Svensson arrangement, the pattern directly on the ground glass. The small errors introduced by the uncorrected cylindrical lens are of no consequence in such a visual inspection and may be eliminated by shifting to the scanning process in obtaining the permanent photographic records of an experiment.

In using the cylindrical lens method the straightedge, D (Figure 11), is replaced by a diagonal slit as shown at A in Figure 13, a. Although not visible in this figure, two pins in the groove at E aid in fixing the position of the diaphragm.

The angle of the slit is adjusted by rotating the disk, B, in the frame, F. Adjustment of the length of the slit may be made by moving the inserts, I and I', with the aid of the racks and pinions. As shown in Figure 13, b, which is a diagram of the disk, B, with the inserts removed, the slit width is controlled by turning the knob, K, and is given directly on the scale at S.

With many boundaries a slit of uniform width gives a pattern, such as that of Figure 14, a, for example, in which the base line and top of the peak are broad. The appearance of the pattern can be improved, as suggested by Svensson (*16*) and illustrated in Figure 14, b, by tapering the ends of the diagonal slit where it intersects the undeviated image of the horizontal slit and also that having the maximum deviation. (It has not been demonstrated that this device does not distort the pattern by shifting the base line, for example, relative to the remainder of the curve.) The inserts, I and I' (Figure 13, a), are constructed, therefore, as shown in Figure 13, c. In this enlarged diagram of the underside of one of the inserts, the gear sectors, G and G', are flush-



a b

Figure 14. Cylindrical Lens Pattern of a Single Boundary

a. θ = 45°, 1-mm. slit with parallel edges
b. θ = 45°, spindle-shaped slit

mounted. As suggested by Burns and Henke (*2*) the edges of the taper are made co-planar by having the sectors move in an annular channel, *C*, that is concentric with the apex of the taper. When the insert is in position in the assembled slit (Figure 13, *a*), the sectors may be adjusted independently by turning the slotted heads, *H* and *H'*, to which the pinions, *J* and *J'* (Figure 13, *c*), are attached. Since the edges of the leaves, *L* and *L'* (Figure 13, *b*), that form the parallel sides of the slit are beveled underneath while those of the gear sectors are beveled from above, all six edges of the spindle-shaped slit are co-planar. Moreover, the plane of the slit is identical with that of the straightedge with which it is interchangeable, so that it is not necessary to refocus the illuminated horizontal slit on making this change.

Since the cylindrical lens is not used in the scanning procedure, it is mounted so that it may be readily placed in, or removed from, the optical system. As shown in Figure 15 this lens, *C*, is held in a frame, *F*, that pivots on a shaft at *A*. When this assembly is inserted through a rectangular opening in the camera tube, as indicated at *N* (Figure 6), the curved plate, *P* (Figure 15), overlaps this opening and serves to support the lens and to exclude stray light. With the frame, *F*, in contact with the stop, *S*, the face of the lens is normal to the axis of the camera. The diameter of the camera tube is such that rotation, by means of the knob, *K*, of the cylinder lens through 90° removes it completely from the path of the image-forming rays. Vertical alignment of the lens is afforded by the small clearance between the ends of the bar, *B*, one of which is visible at *E*, and the sides of the opening in the camera tube. The dimensions of this opening parallel to the camera axis and the overlap of the plate, *P*, are such, however, as to provide a focusing adjustment.

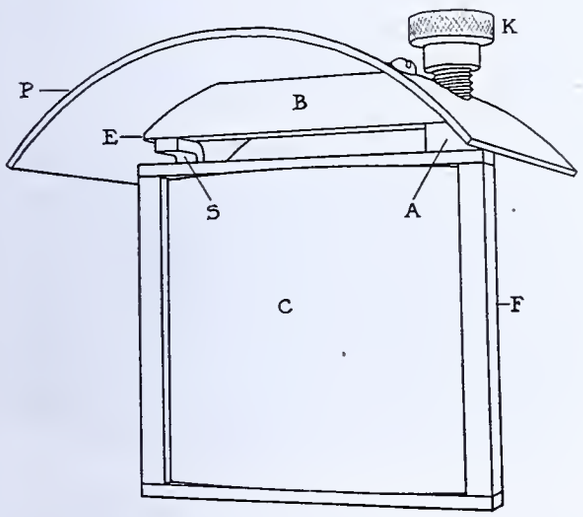


Figure 15. Support for Cylindrical Lens

This completes the description of the optical equipment. Owing to the size of the apparatus it is usually assembled where it is to be used and the responsibility for the alignment and focusing of the components therefore falls upon the investigator. In making these adjustments the chief object is to obtain a sharply defined, vertical base line in the focus of the camera, so that the pattern will be due solely to changes of refractive index in the boundaries and not to aberrations elsewhere in the system. Since imperfect base lines can arise from inadequately corrected or improperly focused lenses, or from schlieren in the windows of the thermostat and moving boundary cell, it is essential to test each of these in turn in order to locate the origin of the distortion, and, if possible, to correct for it. In the tests described below the equipment in the author's laboratory is taken as a typical example.

INSTALLATION OF EQUIPMENT

TESTING AND FOCUSING THE SCHLIEREN LENS. The schlieren camera is well adapted for testing the schlieren lens.

With the water and windows of the thermostat removed, the axes of the camera and schlieren lens are brought into coincidence and the horizontal slit, provided with an appropriate filter, is centered on their common axis. [The axis of a lens may be found by bringing the images of a light source that are reflected from the surface of the lens, four in the case of an air-spaced achromat, into coincidence with the source itself. Campbell (*3*) has described a simple way to do this.]

It is convenient to make the tests with the cylindrical lens method. Both the horizontal and diagonal slits are nearly closed and the latter is set at an almost horizontal angle. Svensson suggests (*17*) $\theta = 85°$. If the lateral magnification factor, *K*, due to the cylindrical lens is 2.05, as in the equipment described here, a vertical displacement, ΔY, at the diaphragm is converted into a lateral displacement on the screen of

$$\Delta N = K \times \tan\theta \times \Delta Y = 24\ \Delta Y \qquad (3)$$

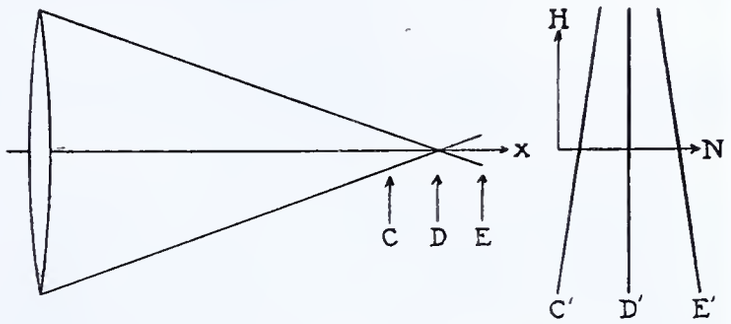


Figure 16. Test for Focus of Illuminated Slit

If the schlieren lens has no spherical aberration, all the (monochromatic) rays come to focus at *D* as indicated in Figure 16. With the diagonal slit at this position a straight vertical line is obtained on the screen as shown at *D'*. Deviations from the vertical become apparent on bringing this line into coincidence with a vertical ruling on the ground glass. Within the limits of error of this test the author's lens is free from spherical aberration. (All the lenses in the electrophoresis equipment at The Rockefeller Institute were made by the Perkin-Elmer Corporation, Glenbrook, Conn.)

A sensitive method for locating the plane, *D*, of the schlieren diaphragm will also be apparent from Figure 16. If, for example, the diaphragm were placed at *C*, the rays from successive horizontal sections of the lens would pass the inclined slit at positions progressively displaced laterally and the line image on the screen, although remaining straight, would now be inclined to the vertical as shown at *C'*. If the diaphragm were placed at *E* the image, *E'*, would have the opposite inclination. In this manner the focal plane of the schlieren lens can be readily located to the nearest millimeter and by shifting the illuminated slit along the axis can be brought into coincidence with the diaphragm.

Figure 16 also serves to illustrate a method for determining the longitudinal chromatism of the lens. Suppose the illuminated slit, provided with a yellow filter, is focused on the diaphragm at position *D*. The yellow line on the ground glass is then straight and vertical. On replacing the yellow by the green filter the straight green line on the screen is now inclined to the vertical as though the diaphragm were at *E* with yellow light. The focus for $\lambda = 5461$ is, therefore, nearer the lens than for $\lambda = 5780$. Since ΔN, the deviation of the green line from the vertical, is 2 mm., $\Delta Y = 0.08$ mm. From similar triangles the displacement of the focus along the axis is $1820\ \Delta Y/100 = 1.5$ mm. The displacement for $\lambda = 4359$ is 3.2 mm. With air in the thermostat the lens is thus undercorrected chromatically. This compensates for the effect of the 30-cm. (12-inch) thickness of water in the image space of the schlieren lens when the thermostat is filled. Under the actual working conditions the chromatic correction of the author's lens is essentially complete. If the windows of the thermostat and cell do not introduce chromatic aberrations, the illuminated slit does not need to be refocused on shifting from one (visible) wave length to another.

In the case of the schlieren lens the only other aberration that needs to be considered is coma (*6*).

The schlieren camera can be used most efficiently if the illuminated horizontal slit, *S* (Figure 2) is placed some 15 to 20 mm. below the camera axis, so that the normal slit image is above that axis. As shown in Figure 17, the peripheral rays then come to focus in the same plane as those through the central portion of the lens but at a different level. The resulting pattern on the screen is shown at *D'* and the deviation from the vertical, ΔN, is a measure of the comatic blurring, ΔY. With the illuminated slit 16 mm. below the axis, ΔN is 2.4 mm., so that $\Delta Y = 0.1$ mm. from Equation 3. By tilting the schlieren lens slightly, so that its

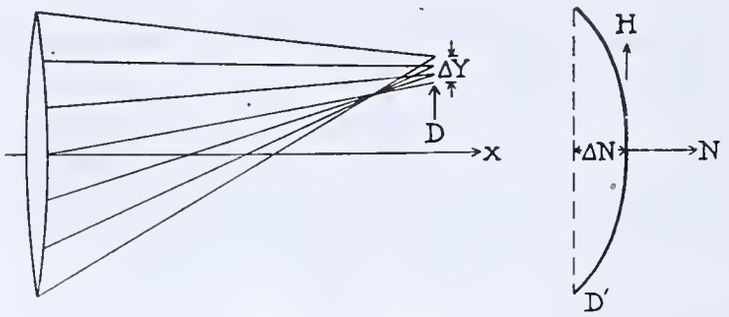


Figure 17. Test for Coma

axis again passes through the center of the horizontal slit, this blurring may be eliminated. It is of no consequence that this axis is now inclined slightly to that of the camera.

Aberrations Due to Windows of Thermostat and Cell. In the equipment described here the thermostat windows are flat to within a few wave lengths. Their introduction into the system does not alter significantly the performance of the schlieren lens if the Bakelite rings in which these windows are clamped are accurately machined. Otherwise the windows may be bowed so as to introduce a form of astigmatism into the system. This is most serious at the windows in contact with the water and is frequently not detectable until the thermostat is filled. If this form of astigmatism is present, the image of the illuminated slit is displaced along the axis as this slit is turned from the horizontal position and is in sharp focus only when the slit is parallel to the axis of the weak cylindrical lens that the bowed window simulates. When viewed, with the aid of the Philpot-Svensson arrangement, on the ground glass of the schlieren camera, astigmatism appears as a blurring of the central portion of the vertical line. If this aberration cannot be eliminated by truing the Bakelite rings, it can be reduced by making the axis of the bowed window horizontal.

Even with vigorous stirring of the thermostat water, localized temperature fluctuations are usually sufficient to be detectable as weak schlieren. The fluctuations are rapid, however, in comparison with the usual exposure interval and thus cancel.

In spite of the progress that has been made in their construction, the windows of the moving boundary cell remain the chief cause for crooked base lines. The optical imperfections in these windows frequently simulate a spherical error in the schlieren lens and may be interpreted with the aid of Figure 18.

Let B, C, and E be the points on the axis at which axial, intermediate, and peripheral rays, respectively, come to focus. With the cylindrical lens method the curves B', C', and E' are the corresponding patterns on the screen. The best position for the diaphragm is at D; in this position the average deviation of the curve from the vertical is a minimum.

In addition to distorting the base line, the imperfections in the cell windows also frequently introduce astigmatism and thus blur this line. The scanning method has an advantage in this regard, since it uses only a narrow vertical section at the center of the channel image where the distortion is usually a minimum.

Focusing the Camera. A 100-mm. precision glass scale (the Zeiss scale No. 126,454 is satisfactory) that may be immersed in water is almost indispensable in the focusing of the camera.

With this scale mounted in the support for the moving boundary cell so that the graduations are in plane C (Figure 10), this support is placed in the thermostat. The approximate position of the object plane for the camera can then be determined by examining the scale image on the ground glass as the support is moved along the optic axis in the thermostat. If, now, a low-power microscope is focused on the ground glass and the image of a scale line examined directly in the microscope after removal of that glass, no difficulty should be experienced in locating the object plane to the nearest 1 or 2 mm. As a check, photographs may be taken with the scale at different positions along the axis in the neighborhood of the object plane and the photographs examined under the microscope. The one with the scale in the object plane will have the lines most sharply defined and, with the aid of a comparator, can be used to determine the enlargement factor, E.

Examination of this scale photograph also indicates that the distortion introduced by the camera objective in The Rockefeller Institute equipment is negligible. Although some comatic blurring of the lines at the edges of the field is apparent, the interval between the lines remains uniform within the limits of error, 5 microns, of the comparator readings. This result might be expected in view of the fact that the camera objective is similar to the schlieren lens and is used at a smaller aperture.

Since it is convenient to have the enlargement factor unity, it is usually necessary to adjust the length of the camera. If the image distance is v' when the enlargement is E and v when $E = 1$, the adjustment is $v' - v = (E - 1)f$, in which f is the focal length of the objective. This follows from the lens formulas, $v/u = E$ and $1/u + 1/v = 1/f$, in which u is the object distance. Since the adjustment is small, f is given with adequate precision by the relation $f = v'/(E + 1)$.

Owing to the convergence of the light after it leaves the schlieren lens, the cell, to be uniformly illuminated, must be placed close to this lens. With the camera adjusted so that $E = 1$ it is moved as a unit along the axis until its object plane coincides with the plane through C (Figure 12). It will also be apparent that these adjustments must precede the focusing of the horizontal slit on the schlieren diaphragm.

Another test for the camera focus, one that is peculiar to the schlieren method and is mentioned by Schardin (*13*), is illustrated in Figure 19, where a series of rays has been traced through a somewhat idealized system.

Since the rays are spaced at equal intervals in the object plane, C, where the incident illumination is uniform, their "density" at any level on a vertical line may be taken as a measure of the light intensity at that level. If a boundary is present in the region from h_1 to h_2, with the maximum gradient at h_m, the rays through this region are then deflected as shown but, in the absence of a diaphragm, the illumination remains uniform in the image plane at G. In a neighboring plane at G', however, the illumination is above the average at the level, H', and below it at H'', whereas this order of intensities is reversed in the plane at G''. Consequently, if a boundary is present in the channel and the diaphragm is removed, the cell image is uniformly illuminated only in the focal plane of the camera.

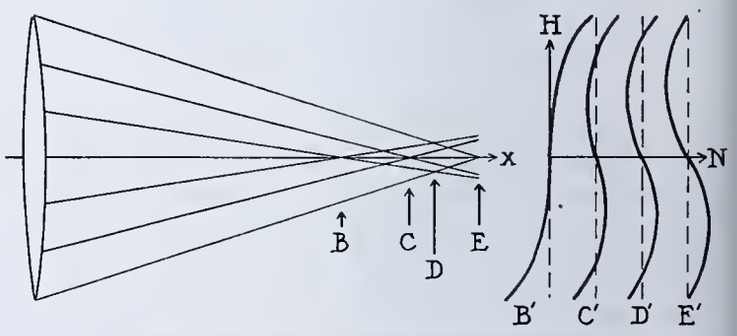


Figure 18. Effect of Spherical Aberration

This test for the camera focus is surprisingly sensitive if the boundary is sharp—i.e., one for which the refractive index difference, Δn, is small and the maximum gradient, n'_m, is large. It is preferable to have n'_m not so large, however, that the lens mount acts as a schlieren diaphragm. With such a boundary in the channel a movement of the ground glass by as little as 1 mm. from the focal plane will produce the effect.

It has already been noted that improper focusing of the horizontal slit on the schlieren diaphragm leads to a pattern with a sloping base line. An improperly focused camera leads, on the other hand, to tilted peaks in the pattern. Thus, if the plate were placed at G' (Figure 19) the maximum gradient in the boundary would appear to be at level H'_m, whereas its true position in the now slightly enlarged image is at H_m. Since the displacement,

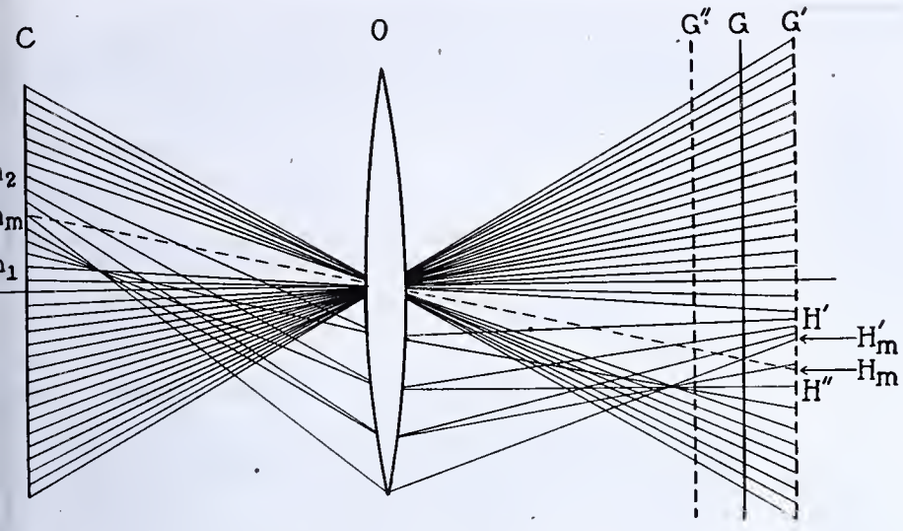


Figure 19. Test for Camera Focus

$H'_m - H_m$, is proportional to the deflection the ray undergoes in he boundary, the resulting peak in the pattern is tilted.

The effects of improper focus of the horizontal slit and of the chlieren camera are illustrated in Figure 20. In this example he boundary was due to a 2% raffinose solution diffusing into ater.

In order to demonstrate the symmetry of the gradients in this oundary it was first scanned, as shown in Figure 20, *a*, with the luminated slit and the camera both properly focused. A white ne was then drawn through the maximum gradient parallel to he recorded black reference line. The symmetry about this hite line is apparent. Moreover, this symmetry was not lost hen the boundary was next scanned after moving the illumiated slit 10 cm. toward the thermostat, Figure 20, *c*. In this gure, however, the sloping base line, due to the improperly fo-used slit, is evident. Finally, the boundary was photographed ith the slit in focus but with the cell moved 10 cm. toward the amera (Figure 20, *b*). Here the base line is horizontal but the ilting of the peak, because the camera is no longer focused on the ell, is apparent from the asymmetry about the white line that s drawn as in *a* and *c*. Although some diffusion occurred during he recording of these patterns, the lower maximum in *b* is due nainly to the shorter distance from the cell to the diaphragm.

Focusing the Cylindrical Lens. Although the use, in the Philpot-Svensson arrangement, of an achromatic cylinder lens would be desirable, only simple lenses have thus far been em-ployed. The one used by the author is a 75 × 75 mm. plano-cylindrical lens with a focal length of 416 mm. Since it is free rom a defect sometimes encountered in lenses of this type—namely, the superposition of a spherical component on one of the aces—its introduction into the system does not appreciably hift the image plane of the camera. This lens may be aligned nd focused as follows:

If a contact print of the glass scale is made on a photographic late the rulings will appear in the print as transparent lines on an paque background. If this print is then placed in the plane of he schlieren diaphragm so that the rulings are vertical, the ntersection of each ruling with the image of the illuminated orizontal slit becomes essentially a point source of light in the bject plane of the cylindrical lens. Since a point in the object lane of such a lens is brought to focus in the image plane as a ine parallel to the axis of the cylinder, a grid of parallel lines will ppear on the ground glass of the camera. By rotation of the amera tube the axis of the cylindrical lens, and hence the lines n the screen, can be made vertical. As in the case of the camera bjective, this lens may be focused by observing these lines, with the aid of a microscope, as it is shifted along the camera axis. The aberrations introduced by this uncorrected lens will be apparent, however, in that no setting can be found in which all the lines are uniformly sharp.

Blurring due to the lack of correction in the cylindrical lens is not to be confused with that which arises when this lens is not normal to the camera axis. The vertical alignment is not critical but small deviations of the cross-axis of this lens from the normal lead to a blurring that becomes progressively worse from one line to the next. A setscrew in the stop at *S* (Figure 15) is provided for making this adjustment.

The errors introduced by the cylindrical lens are due chiefly to the uncertainty in the position of a blurred line. The magnitude of this uncertainty may be obtained by photographing the grid with the slit image at the axis and 15 mm., say, above and below the axis, respectively. The spacings, at different levels, between the lines on each of the three plates are then determined and are found, in the case of the lens used by the author, to be 10.24 mm. with an average deviation from the mean of 0.01_6 mm. The maximum deviations that occur near the edge of the field are as great as 0.06 mm., however, and, until corrected cylindrical lenses become available, render this method unsuitable for precise work. Since the spacing of the points in the real scale is 5 mm., the lateral magnification, *K*, is 2.048.

Determination of Constants *a* and *b* of Equation 1. As is clear from Equation 1, $\Delta Y = abn'$, the dimension, *a*, of the channel parallel to the optic axis and the optical distance, *b*, from the center of that channel to the schlieren diaphragm should be known with a precision equal to that with which the slit image displacement, ΔY, can be determined. Under favorable conditions this displacement can be measured to 0.02 mm. and if it has the maximum value of about 4 cm. permitted by the camera the corresponding error is 0.05%. This precision in the determination of *a* and *b* may be obtained as follows:

The value of *a* near the top and bottom of the channel is determined to 0.01 mm. with the aid of an inside vernier. The following procedure, suggested by D. A. MacInnes, may be used at other levels.

With the center section of the cell in a horizontal position a brass bar whose length, *l*, is slightly less than *a*, and whose ends are flat and parallel, is inserted in the channel with one end resting on what is now the bottom window. With the aid of the micrometer adjustment the difference, $a - l$, is determined by focusing a microscope alternately on the upper end of the bar and on dust particles on the inside wall of the top window. This is repeated as the bar is shifted along the channel. The dimensions of the bar used by the author are 2.475 × 0.95 × 0.27 cm. In this manner the values 2.509, 2.508, 2.507, 2.507, 2.506, 2.506, and 2.507 cm. were obtained at equally spaced intervals along a typical channel. Since the average deviation from the mean value of 2.507 is only 0.03%, *a* may be taken as independent of *h* in this instance. The value of *a* for the other side of the channel in the same cell was also essentially constant and equal to 2.512 cm.

One method for determining the optical distance, *b*, is illustrated in Table I. In the first column is listed each successive

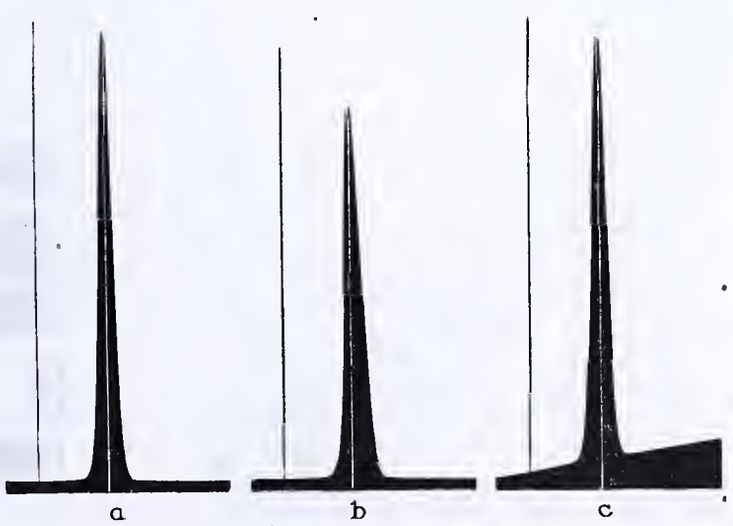


Figure 20. Effect of Improper Focusing of Illuminated Slit and Camera

Table I. Determination of the Optical Distance, b

Element	Δx	n	$\Delta x/n$
$a/2$	1.25	1.334	0.94
Cell window	0.30	1.526	0.20
Cell window to inside thermostat window	23.7$_7$	1.334	17.8$_2$
Inside thermostat window	0.63	1.526	0.42
Air space in double window	2.54	1.000	2.54
Outside thermostat window	0.63	1.526	0.42
Outside thermostat window to diaphragm	159.7$_2$	1.000	159.7$_2$
		b =	182.0$_6$ cm.

element in the path while its dimension, parallel to the optic axis, is given in the second column. The factor, b, is then the sum of these distances after division of each by the corresponding refractive index, column 3, referred to air as unity.

It is also convenient to determine b with the aid of a small glass prism. With the prism oriented for minimum deviation (*5*), it is placed in the object plane of the camera—i.e., C (Figure 10)—and forms at the schlieren diaphragm an image of the illuminated slit that is displaced ΔY cm. below the normal slit image. A crown-glass prism of about 6-diopter power is suitable if the prism angle, A, and the minimum deflection in air, D'', for λ = 5769–5790 are known to within 2″ of arc. (Prisms of this type, acceptable for calibration by the Bureau of Standards, Washington, D. C., may be obtained from the Maberg Optical Co., New York, N. Y., and from the Perkin-Elmer Corporation, Glenbrook, Conn.) The formula $n_G = \sin {}^1/_2 (A + D'')/\sin {}^1/_2 A$, may then be used to compute n_G, the refractive index of the glass. Since the refractive index of water, n_0, is 1.33431 (*18*) at 0.5°, whereas n_G is essentially the same as at room temperature, the deflection in water, D', is $D' = -A + 2 \sin^{-1} (n_G \sin {}^1/_2 A/n_0)$. After refraction at the water-air interface the deflection, D, outside of the thermostat is $D = \sin^{-1} (n_0 \sin D')$ and $b = \Delta Y/D$. The value of b obtained in this manner, 182.0$_2$ cm., is in good agreement with that measured directly, 182.0$_6$ cm., Table I, and is probably the more reliable since the prism method tends to compensate for imperfections in the windows.

Measurement of Refractive Index Differences. In order to minimize the disturbing effects of convection in the electrophoresis of protein solutions, and also to reduce the growth of microorganisms, most work on these solutions is done near 0° C. The quantitative interpretation of the patterns has been hampered, however, by the lack of precise refractive index measurements on solutions at this temperature. When used in conjunction with hollow prism cells the optical equipment described here is well adapted for such measurements and this report concludes with a description of this application of the apparatus.

Since the hollow prism is immersed in water no deflection, except that due to imperfections in the prism windows, is observed when it is also filled with water. The deflection that occurs when a dilute aqueous solution is introduced into the prism cell is due to the difference, Δn, between the refractive index of the solution, n_s, and of water, n_0. The method is thus a differential one.

As in the determination of the optical distance, b, with the aid of the glass prism the relations are

$$n_s/n_0 = \sin {}^1/_2(A + D')/\sin {}^1/_2 A \quad (4)$$

and

$$\sin D = n_0 \sin D' \quad (5)$$

where A is now the refracting angle of the hollow prism, D' is the minimum deflection in water due to the aqueous solution of refractive index, n_s, and D is the deflection after emergence from the thermostat. Equation 4 may also be written

$$n_s/n_0 = (\sin {}^1/_2 A \cos {}^1/_2 D' + \sin {}^1/_2 D' \cos {}^1/_2 A)/\sin {}^1/_2 A = \cos {}^1/_2 D' + \sin {}^1/_2 D' \cot {}^1/_2 A$$

With sufficiently dilute solutions D' is small, $\cos {}^1/_2 D' = 1$, $\sin {}^1/_2 D' = {}^1/_2 D'$ and Equation 5 becomes $D = n_0 D'$, so that

$$(n_s - n_0)/n_0 = \Delta n/n_0 = ({}^1/_2 \cot {}^1/_2 A) D'$$

or simply

$$\Delta n = ({}^1/_2 \cot {}^1/_2 A) D = ({}^1/_2 \cot {}^1/_2 A)\, \Delta Y/b \quad (6)$$

In the case of the two prism cells shown diagrammatically in Figure 21, A = 60° 36′ and 30° 8′, respectively, and the corresponding cell constants, ${}^1/_2 \cot {}^1/_2 A$, are 0.8556 and 1.857. These angles were determined with the aid of a spectrometer of ordinary precision after filling the cells with mercury. For an accuracy of 0.05% in the cell constant a 60° angle needs to be known to the nearest 90″ and a 30° angle to the nearest 50″. With the 60° cell an uncertainty of 0.02 mm. in ΔY corresponds to an error of 1×10^{-5} in Δn and the precision of the method thus compares favorably with that of the other available procedures (*1*). It may also be shown that within this limit of error the approximate Equation 6 is valid if ΔY does not exceed 2 cm. With a 60° cell and b = 182 cm. this corresponds to a 5% protein solution. Similarly, if the bisector of the refracting angle is vertical, the error introduced by the difference between the actual deviation and the minimum deviation will not exceed 1×10^{-5} in Δn.

As is shown in Figure 21, *a* and *b*, the two prism cells are supported in such a way as to be interchangeable with the Tiselius cell at C (Figure 10). Although the 60° cell is ordinarily used for dilute solutions and the 30° cell for more concentrated ones, the scanning photograph (Figure 21, *d*) was obtained with the same solution in both cells in order to illustrate not only the experimental procedure but also a test of the method. The solution in this instance was approximately 1 N barium chloride while Figure 21, *c*, was recorded on the same plate with water in the cells. The print (negative) is mounted here so that the height, H, corresponds to that at the cells. The gaps between the exposed areas are due to the opaque cemented joints of a cell.

The right-hand edge of each exposed area in Figure 21, *c* and *d*, is the value of N as a function of H. The displacement of this edge at the level of a cell from that above or below is proportional to the slit image displacement, ΔY, at the schlieren diaphragm, the factor of proportionality being the ratio, M, of the plate movement to that of the diaphragm in the scanning process. If the system were optically perfect and the water in the cells identical with that in the thermostat, the right-hand edges in *c* would all lie on a vertical line. Since this ideal condition is not realized, especially with the 30° cell, correction must be made by subtracting the displacement that occurs with water in a cell from that when solution is present. With the aid of a two-coordinate comparator the value of N in *c* at some level conjugate to the 30° cell, say H_{30}, is determined relative to a value of N outside the cell, say at H_0, as zero. This value of N may be designated N_0^{30}. In Figure 21, *d*, the value of N at the same level, H_{30}, relative to that at H_0, is also determined and is designated N^{30}. The difference, $N^{30} - N_0^{30}$, is then equal to $M\,\Delta Y$ and leads, in the present example, to $\Delta n = 0.01574_3$. Similar measurements at some level conjugate to the 60° cell, say H_{60}, give the closely agreeing value of $\Delta n = 0.01574_3$, thus indicating that the effect of varying the refracting angle, A, is given correctly by Equation 4.

Accidental errors in the location of the edge can be reduced by taking the average of readings at values of H conjugate to other levels both inside and outside a cell. Although the water with which a prism cell is filled in determining N_0 should be the same as that used in preparing the solution, the purity of the thermostat water, on the other hand, is not critical.

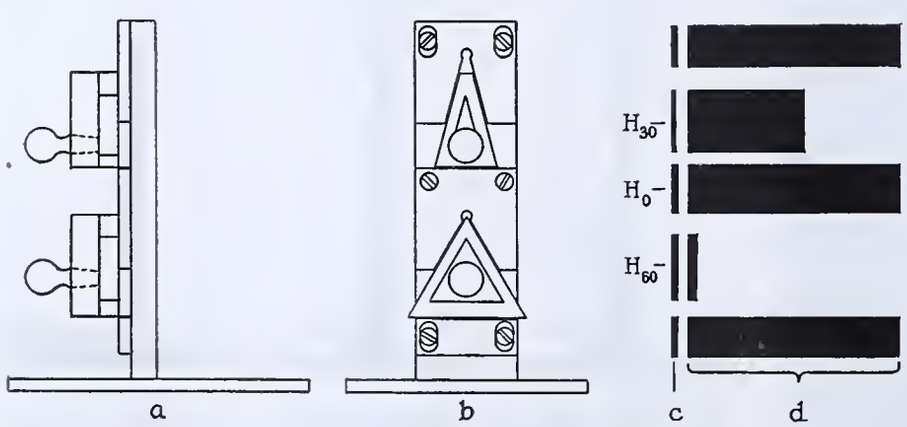


Figure 21. Determination of Differences of Refractive Index

Owing to imperfections in the optical system both the normal and deviated slit images are less sharply defined than the illuminated slit itself. If the slit image displacement were measured directly with a micrometer ocular, say, this blurring represents a source of uncertainty that is eliminated with the schlieren scanning camera since this permits, in effect, the use of images formed by selected, small portions of the field. The prism cell specifications are thus not so exacting as in the conventional spectrometer methods.

While the use of the cylindrical lens method for the direct determination of Δn cannot be recommended, it can be used to advantage to determine the dispersion due to the solute—i.e., the differences $\Delta n(\lambda_1) - \Delta n(\lambda_2)$, in the refractive index increment for different wave lengths. With a solution of sufficient concentration in the prism cell to give a good deflection, the horizontal and inclined slits are both nearly closed, the filter is removed, and the separation of the lines of the mercury spectrum in the focus of the schlieren camera is measured with the aid of a micrometer ocular. If Δn for the yellow lines, say, has been determined independently by the scanning procedure, the values of Δn for the red, green, blue, and violet lines can then be estimated with adequate precision from the respective separations between these and the yellow lines.

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Deproteinization by Metaphosphoric Acid

A Review of Its Value for the Analyst

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A review of the literature on deproteinization by metaphosphoric acid is presented. Metaphosphoric acid is accepted as the most generally reliable deproteinizing agent for determining ascorbic acid in plant and animal materials, and is of value for determining lactose in milk and deproteinizing clinical specimens. It can also be used for isolation and quantitative determination of protein in agricultural and other products.

WHILE there exists today a need for a reliable deproteinizing agent for biological specimens in general which would be readily available, easy to handle, and free from objectionable effects on the constituents to be determined in the filtrate, comparatively little attention has been given to the valuable properties of metaphosphoric acid and its sodium salts, except for ascorbic acid determination.

Although a large number of publications dealing with deproteinization by metaphosphoric acid are to be found in foreign journals, there is a scarcity of coordinated authentic information on metaphosphoric acid and metaphosphates

EARLY WORK

While metaphosphoric acid, HPO_3, can be prepared by combining phosphorus pentoxide with water or with orthophosphoric acid, H_3PO_4, the product is a highly viscous fluid, impractical for use. Where investigators mention metaphosphoric acid without giving particulars, they refer usually to the so-called glacial phosphoric acid which is commercially available in the form of sticks. This is an acid sodium phosphate glass approximating in composition the formula $Na_2O.H_2O.2P_2O_5$. Graham's salt, first obtained in 1833 by fusing and quickly cooling sodium dihydrogen phosphate, is a substantially neutral sodium phosphate glass represented by the formula $Na_2O.P_2O_5$. This glass is commonly though inaccurately designated as "sodium hexametaphosphate". When devitrified by heating below 625° C., it yields crystalline water-soluble sodium trimetaphosphate, $(NaPO_3)_3$. Other sodium phosphate glasses with ratios of sodium oxide to phosphorus pentoxide of up to approximately 1.7 may be made.

The complexity of the phosphate glasses is illustrated by the finding of Karbe and Jander (*24*) that, when Graham's salt is prepared at a temperature of 1160° C., the molecular weight of the anion amounts to more than 7000.

Berzelius (*3*) discovered in 1816 that a phosphoric acid, known today as metaphosphoric acid, has the property, not shared by either the pyro- or the orthophosphoric acid, of coagulating egg white. In 1825 Engelhart (*12*) presented data on the precipitation of dilute blood serum proteins with metaphosphoric acid. In 1880 Lorenz (*31*), experimenting with dilute solutions of gelatin, obtained precipitates by means of metaphosphoric acid or of sodium metaphosphate and hydrochloric acid. Repeated washing with water was found to reduce the phosphorus pentoxide content of the gelatin-metaphosphate compound. No antiseptic

was required during the washing operation, since the compound is resistant to bacterial decomposition.

In 1888 Lieberman (*29*) obtained precipitates of egg albumin with glacial phosphoric acid, which resembled nucleins. A year later Pohl (*43*) treated dilute serum albumin with concentrated sodium metaphosphate and obtained upon acidification with hydrochloric acid a precipitate with a nearly constant phosphorus content (5.54 to 5.69%). Hemialbumoses, prepared from Witte's peptone by means of precipitation with ammonium sulfate, also gave precipitates with metaphosphoric acid, which dissolved on heating and reappeared on cooling.

In 1892 Malfatti (*32*) obtained precipitates by treating blood serum proteins, dissolved in 0.3% hydrochloric acid, with metaphosphoric acid. The precipitates were found to contain 6.1% phosphorus; after repeated (five times) dissolving in ammoniated water and reprecipitation with acetic acid, the phosphorus content was reduced to 1.6%. From the filtrates a product rich in phosphorus was obtained upon acidification with hydrochloric acid.

The following year Denigès (*9*) recommended a 5% solution of sodium metaphosphate with some hydrochloric or sulfuric acid for deproteinization of milk for lactose determination. Five years later Chassaigne (*5*) used sodium metaphosphate solution and hydrochloric acid as deproteinizing agent in his studies on the reducing substances of blood.

Pick (*42*) studied in 1898 the precipitating effect of metaphosphoric acid on aqueous solutions of various albumoses and peptones, and obtained only a trace of turbidity with protalbumose, and with the secondary albumose A a white precipitate, which dissolved on heating and reappeared on cooling. None of the other albumoses, or the peptones, gave any turbidity.

In 1902 Fuld (*16*), who took up Hofmeister's work on protein-metaphosphate compounds, reported earlier (1896) work by Georgiewsky from the same laboratory, who precipitated solutions of serum albumin with an excess of glacial phosphoric acid, as well as with solutions of phosphate glass (Graham's salt) and hydrochloric acid, and obtained products with nearly constant phosphorus content, averaging 3.47% phosphorus, which remained practically the same even after dissolving in ammonia followed by reprecipitation by acid. Fuld also showed the solubility of the protein-phosphate compound in alkalies and neutral salts, a subject which has been investigated in recent studies by Perlmann and Herrmann (*40*), Briggs (*4*), and Horvath (*23*).

In his own experiments, Fuld (*16*) precipitated 12.5% solutions of serum albumin with increasing amounts of 10% glacial phosphoric acid, and obtained products with a nearly constant phosphorus content (average 3.33%). Fuld interprets his lower figure, as compared with Georgiewsky's, as due to more thorough washing of the precipitate, and the prevention of the splitting off of albumoses by the acid by immediately pouring large volumes of water on the precipitate. With crystalline ovalbumin Fuld obtained under similar conditions a product containing 2.43% phosphorus, and with Hamarsten's casein an average of 3% phosphorus. No constant figures were obtained with crystalline hemoglobin (2.83 to 4.06%), owing to the gradual splitting off of hematin. Serum globulins gave unsatisfactory results. Protalbumoses showed turbidity, which dissolved on heating or in excess acid. Peptones gave no turbidity. Fuld also established the fact that the amount of metaphosphoric acid combined by a protein depends on its diaminoacid content, and is suggestive of an amino group link, a fact which has been corroborated in recent times by other investigators (*4*, *39*, *40*).

In his 1914 study on glucose and lactic acid of blood and other body fluids, Chelle (*6*) used sodium metaphosphate (phosphate glass, 0.6% solution) as deproteinizing agent in the presence of sulfuric acid.

DEPROTEINIZATION OF BLOOD

In 1916 there appeared the substantial paper by Folin and Denis (*13*) on deproteinization of blood and urine by glacial phosphoric acid, which is said to be remarkably effective as a precipitant for the blood proteins. It is better than colloidal iron and fully as good as trichloroacetic acid, and yields filtrates which are as colorless and clear as water, which remain clear, and which boil without foaming.

The recommended procedure is to dilute 5 cc. of blood with an equal volume of water, and to precipitate the proteins by 3 cc. of 25% metaphosphoric acid. The solution should be prepared fresh every several days, as a gradual reversion to orthophosphoric acid takes place. The mixture is diluted to 50 cc. and, after shaking, filtered through a dry filter paper. The technique of nonprotein nitrogen determination in the filtrate is given in detail. For urea determination the urease effect must be completed before the metaphosphoric acid is added. For deproteinization of urine 10 cc. are treated with 1 cc. of 25% metaphosphoric acid. The procedure of ammonia determination as well as of urea in urine by the nesslerization method is fully described.

In the following year, Sjollema and Hetterschy (*46*) used glacial phosphoric acid for deproteinization in the determination of nonprotein nitrogen in dog and ox blood, as well as in horse blood serum, by a modified Bang's technique. The specimens were absorbed on strips of filter paper, weighed, immersed for 1 hour in 3.5% metaphosphoric acid and washed, and the nonprotein nitrogen was determined in the fluid. The authors also conducted parallel deproteinization experiments by phosphomolybdic and trichloroacetic acid. The figures of nonprotein nitrogen in the filtrates were highest for trichloroacetic acid, next for metaphosphoric acid, and lowest for phosphomolybdic acid. Differences disappeared when dialyzed blood was used, where a part of polypeptides, amino acids, etc., were removed. Phosphomolybdic acid gives a precipitate in metaphosphoric acid filtrates.

In 1921 Wolff (*50*), using Bang's technique, found that metaphosphoric acid precipitates only a small part of primary albumoses, leaving 70 to 80% of them in the filtrate. In it are to be found also over 80% of secondary albumoses and all of the peptones. Phosphomolybdic acid, on the other hand, completely precipitates the primary albumoses and leaves only 8% of the secondary albumoses in the filtrate. Peptones are precipitated by it very incompletely.

Wolff conducted parallel precipitation of blood specimens by phosphomolybdic and metaphosphoric acids, and stated that the difference in the nitrogen contents of the filtrates represented approximately the nitrogen of albumoses. By means of such a technique a large number of clinical blood samples were investigated, showing the existence of a distinct hyperalbumosemia (of 90 to 240 mg. % as against 20 to 65 mg. % in normal individuals) in typhoid fever, pneumonia, and pleuritis. In leukemia, the existing blood hyperalbumosemia originates from the white cells which are extremely rich in albumoses. A light hyperalbumosemia was observed often in cancer. Blood hyperalbumosemia was absent in Bright's disease and patients with stomach ulcers and liver disease.

In the same year Guillaumin (*21*) compared the deproteinization of blood plasma by tungstic, metaphosphoric, and trichloroacetic acids, and found that the average minimal required acidity was a pH of 4.8 at a dilution of 1 to 10. With increasing acidity the nonprotein nitrogen of meta- and tungstic acid filtrates declines, while the trichloroacetic acid filtrate is not much affected by it.

Further comparative studies along these lines were conducted in 1922 by Hiller and Van Slyke (*22*) on ox blood, deprived of its urea nitrogen by means of urease and acration. Results:

	NPN *Mg. %*	Amino N *Mg. %*	Peptide N *Mg. %*
Tungstic acid	28.1	9.2	4.1
Picric acid	...	8.3	4.6
Metaphosphoric acid	28.3	7.9	3.9
Trichloroacetic acid (2.5%)	28.8	7.9	7.0
Colloidal iron	29.4	7.8	...
Trichloroacetic acid (5%)	26.7	7.5	4.9
Trichloroacetic acid (10%)	26.1	7.1	4.6
Alcohol	18.2	4.9	1.4

Investigations by Grigaut (*19*), published during the same ear, were conducted on human whole blood, red corpuscles, and erum, using the following technique for deproteinization by ietaphosphoric acid:

	Cc.		Cc.
erum	10	Whole blood (or red cells)	10
odium metaphosphate (5%)	8	Sodium metaphosphate (5%)	25
ydrochloric acid (2 *N*)	2	Hydrochloric acid (2 *N*)	5

In parallel experiments in deproteinization by trichloroacetic cid equal volumes of blood serum and of 20% trichloroacetic cid were used. The blood serum of patients with chronic ephritis and with diabetes showed a twofold and fourfold, re-pectively, higher nonprotein nitrogen value in metaphosphoric cid filtrates as compared with trichloroacetic acid filtrates, hile the uric acid values were practically identical for both pre-ipitants.

In further studies on the deproteinization of blood, spinal luid, and pathological fluids by metaphosphoric acid, Grigaut nd Zizine (*20*) pointed out the importance of proper dilution nd acidity. The higher the preliminary dilution of the speci-nen, the lower is the nonprotein nitrogen of the filtrate. Dilu-ions of various pathological blood sera 1 to 1 gave much higher onprotein nitrogen figures in the filtrates than a dilution 1 to 0. As to the proper acidity, there exists for every serum an ndividual range of complete deproteinization between the so-alled minimal and maximal acidity. Expressed in cubic cen-imeters of hydrochloric acid (2 *N*) per 10 cc. of serum, the ranges re 0.5 to 4.0 cc.; 0.9 to 2.8 cc.; 0.6 to 4.3 cc.; 1.2 to 3.5 cc.; .7 to 4.3 cc.; and 0.6 to 3.6 cc. A medium acidity and a dilution f 1 to 1 were used to obtain in the metafiltrate certain substances f the polypeptide type less complex than albumoses and peptones, hich are of interest in pathological sera. The techniques used ere:

	Cc.		Cc.		Cc.
erum	10	Whole blood	10	Cerebrospinal fluid	10
Vater	6	Water	20	Sodium metaphosphate (20%)	0.5
odium metaphosphate (20%, approximately 2 *N*)	2	Sodium metaphosphate	5	Hydrochloric acid (2 *N*)	0.5
Hydrochloric acid (2 *N*)	2	Hydrochloric acid (2 *N*)	5		

In a number of pathological sera metaphosphoric acid (1 to 1) ave a higher nonprotein nitrogen than trichloroacetic acid 1 to 1), which is evidently not due to hydrolysis. The fil-rates from metaphosphoric acid were found to contain uro-iline, while the trichloroacetic acid filtrates were free of this onstituent, a phenomenon which is attributed by the authors to ts being bound to protein. Blood filtrates from metaphosphoric cid give urea and uric acid figures similar to the ones from tri-hloroacetic acid, and are a suitable substitute for tungstic acid iltrates for blood sugar determinations by the Folin-Wu method.

During the same year Cristol (*7*) confirmed Grigaut's data hat the metaphosphoric acid filtrates of blood serum contain nore nonprotein nitrogen than the trichloroacetic acid filtrate, nd established by means of a positive biuret test that this ex-ess nitrogen is of protein nature. In Cristol's opinion this extra itrogen in the metaphosphoric acid filtrate may be caused by he splitting effect of the metaphosphoric acid itself, and he was ble to confirm it experimentally, where the metaphosphoric acid vas added either subsequently to the hydrochloric acid or in nixture with the latter (maximal effect), but not where the meta-hosphoric acid was added first. The intensity of the biuret eaction in the metaphosphoric acid filtrates was shown also to e dependent upon the duration of the metaphosphoric acid ac-ion on the serum, increasing with time. By dialyzing blood erum, Cristol was unable to detect any biuret-positive con-tituents in the dialyzing fluid.

In 1923, Cristol and Nikolitch (*8*) made a comparative study f deproteinization by metaphosphoric, tungstic, and trichloro-cetic acids, using the following technique:

	Cc.	
Plasma (or pathological fluid)	10	
1 *N* Sodium metaphosphate (10.2%)	2	Average pH of filtrate 3.3
Water	36	to 3.6 (strong acidity)
1 *N* Sulfuric acid	2	
Plasma	10	
Sodium metaphosphate (1.5%)	10	Average pH of filtrate 4.5
Water	20	to 4.8 (minimal acidity)
0.1 *N* sulfuric acid	10	
Red blood cells (diluted 1 to 1)	10	
1 *N* sodium metaphosphate (10.2%)	10	Average pH of filtrate 2.0
1 *N* sulfuric acid	10	to 2.2 (strong acidity)
Red blood cells (diluted 1 to 1)	20	
Sodium metaphosphate (1.5%)	30	Average pH of filtrate 4.5
Water	14	to 4.8 (medium acidity)
0.1 *N* sulfuric acid	36	

In deproteinization by tungstic acid the minimal and strong acidities of the plasma filtrates (diluted 1 to 5) were approximately the same as for metaphosphoric acid. The filtrates (diluted 1 to 1) from trichloroacetic acid had as minimal acidity a pH of 2.0, and as strong acidity a pH of below 1. In the metaphosphoric and tungstic acid filtrates higher nonprotein nitrogen results were obtained at higher pH values. At high acidity, the metaphosphoric acid filtrates gave the highest nonprotein nitrogen figures from the three precipitants tested. At weak acidity the results were not constant, but for the red blood cells trichloroacetic acid gave the highest nonprotein nitrogen. Cristol and Nikolitch (*8*) were also able to confirm the findings by previous investigators that in metaphosphoric acid filtrates the nonprotein nitrogen is the smaller the higher the preliminary dilution of the specimen, and that these filtrates give a positive biuret test, indicative of "large polypeptide molecules" (wording of the authors) while the test for albumoses and peptones by the Tanret reagent is negative. These polypeptides, which are precipitated from the metaphosphoric acid filtrates by phosphotungstic acid, are believed by the authors not to be present in the blood in free form, but to be split off by the action of metaphosphoric acid from proteins which have become labile under certain pathological conditions. The authors believe that this increase in nitrogen in metaphosphoric acid filtrates may prove of value from a clinical point of view, and they suggested for such a condition the term "index of lability".

During the same year, Minich (*34*) conducted a comparative study on the deproteinization of oxalated horse blood by various precipitants. The deproteinization by metaphosphoric acid was conducted by the method of Folin and Denis (*13*). Minich obtained the following average figures for nonprotein nitrogen in the filtrates:

	N, Mg. %
Trichloroacetic acid	31.03
Phosphomolybdic acid	27.04
Uranyl acetate	21.85
Phosphotungstic acid	18.58
Metaphosphoric acid	33.25

Metaphosphoric acid gave the highest figures, with trichloroacetic acid following. These results were comparable to the ones obtained by the same author on human blood.

Two years later, Mendel and Goldscheider (*33*) used metaphosphoric acid as a deproteinizing agent in the determination of lactic acid in blood. To 1 cc. of the latter were added 6 cc. of water and 1 cc. of glacial phosphoric acid (5%).

In 1928 Balarev (*2*), experimenting with the precipitation of equal portions of albumin by various metaphosphates acidified with acetic acid, determined the amount of residual metaphosphate in the filtrates.

	Normality of Metaphosphoric Acid Added	Normality of Metaphosphoric Acid in Filtrate
Trimetaphosphate (Tamman's)	$\frac{1}{40}$	$\frac{1}{200}$
Tetrametaphosphate (Tamman's)	$\frac{1}{480}$	$\frac{1}{2400}$
Metaphosphate obtained by heating microcosmic salt	$\frac{1}{2500}$	$\frac{1}{12,500}$
Metaphosphoric acid by dissolving P_2O_5 in water	$\frac{1}{5600}$	$\frac{1}{28,000}$

In 1935 Fujita and Iwatake (*15*) successfully used glacial phosphoric acid (5% solution) for deproteinization of ground organs in the determination of reduced glutathione. Trichloroacetic acid was found unsuitable for this purpose because of the presence of ferric iron in the filtrates, which caused an error by liberating iodine from potassium iodide.

VITAMINS, PROTEINS IN AGRICULTURAL PRODUCTS

The following year there appeared a paper by Musulin and King (*36*) on the use of metaphosphoric acid in the extraction and titration method for vitamin C. It was found that metaphosphoric acid in approximately 2% concentration serves to protect vitamin C in solution against atmospheric oxidation, even in the presence of added copper, and also exerts a protective action against oxidation in the presence of trichloroacetic acid. The rate of reaction with 2,6-dichlorophenol indophenol was not appreciably affected by the presence of metaphosphoric acid. A procedure, including the presence of 2% metaphosphoric acid, was recommended for work with both plant and animal tissues.

The same year Penau and Guilbert (*33*) recommended the use of sodium metaphosphate plus sulfuric acid for deproteinization of blood serum in the determination of the activity of lipase and esterase.

In 1939, Knight, Dutcher, and Guerrant (*25*) successfully used metaphosphoric acid in the determination of vitamin C in milk. To 10 cc. of the latter were added 10 cc. of a 10% solution of sodium metaphosphate, acidified with 0.6 cc. of concentrated hydrochloric acid, with immediate and complete flocculation of protein at a pH of 2.5 to 3.0. The vitamin C was subsequently determined in the filtrate.

In 1939–40 several U. S. patents were granted to Fortune (*14*) on a blood sugar test, urine albumin test, and a test for aminobenzene compounds in body fluids, where the deproteinization is done by "sodium hexametaphosphate" at a pH of approximately 4.5. (While the designation "sodium hexametaphosphate" has frequently been applied to the sodium phosphate glass commercially available under the name of Calgon, this product actually has a molar ratio of Na_2O to P_2O_5 of slightly greater than 1 to 1.)

For blood sugar determination one drop of blood is diluted with about 10 cc. of water, and the protein removed by precipitating capsules consisting of:

"Sodium hexametaphosphate"	1/4 grain
Oxalic acid, anhydrous	1/4 grain
Talc, powdered	2 grains

The albumin test in urine requires no heating, and is conducted on 4-cc. portions of urine, diluted with an equal volume of water, by adding a tablet consisting of:

"Sodium hexametaphosphate'	50 mg.
Glutamic acid hydrochloride	50 mg.
Dextrin	2 mg.

After shaking, the cloudiness is directly compared with a turbidity chart.

In the test for aminobenzene compounds in body fluids the following protein-precipitating and diazotizing agent (in capsules) is used:

Sodium nitrite	2 mg.
"Sodium hexametaphosphate"	50 mg.
Glutamic acid hydrochloride	30 mg.
Talc	120 mg.

For this test approximately 2 drops of blood are required, diluted 30 or 40 times with water.

In 1939 Pfützer and Roth (*41*), working on the determination of proteins in agricultural products, recommended the precipitation of proteins by means of sodium metaphosphate at a pH of 2.5.

This was attained by means of hydrochloric acid and a citrate buffer obtained by dissolving 21 grams of citric acid in 100 cc. of 2 *N* sodium hydroxide, adding 89 cc. of 2 *N* hydrochloric acid, and diluting with water to 1 liter (pH 2.5). An excess of metaphosphate was shown to cause a partial redissolving of the precipitate in such varied products as barley grain, carrots (fresh), and gelatin. The addition of various amino acids, as well as choline, colamine, and urea, did not affect the figures of the precipitated protein. The determinations were conducted on 0.4-gram (dry basis) fresh samples, suspended in 50 cc. of water in a 70- to 100-cc. centrifuge tube; 2 cc. of a 10% solution of sodium metaphosphate were added, followed by 0.2 cc. of 2 *N* hydrochloric acid and 10 cc. of citrate. After thorough mixing, centrifuging, decanting of the supernatant liquid, and washing with the same phosphate mixture, the nitrogen in the residue is determined by the Kjeldahl method.

The following year, Eidelman (*11*) demonstrated that trichloroacetic acid destroys in 24 hours at room temperature 60 to 81% of synthetic dehydroascorbic acid, and 40 to 56% of the latter in cabbage juice, while on addition of metaphosphoric acid 65 to 100% of dehydroascorbic acid can be recovered.

In 1941 Kuether and Roe (*28*) used glacial phosphoric acid for the deproteinization of blood in the determination of ascorbic acid. During the same year, Krautman (*27*) published two papers on deproteinization of blood and cerebrospinal fluid by means of a reagent, consisting mainly of dilute glacial phosphoric acid and tungstic acid. This reagent can also be stored in dry form and diluted before use. For deproteinization 9 parts of this reagent are added to 1 part of specimen. The filtrates are suitable for the determination of glucose, nonprotein nitrogen, urea nitrogen, and uric acid.

The 1941 paper by Vonesch and Zimman (*49*) describes a photometric method for the determination of ascorbic acid in blood, where the deproteinization is conducted by means of metaphosphoric acid, followed by lead acetate. In another photometric method by Morell (*35*) for the determination of ascorbic acid in plant materials, 25 grams of pulped fresh plant tissues are mixed at high speed with 100 cc. of a 3% solution of metaphosphoric acid for about 2 minutes. The filtrate is buffered to pH 3.6, and to it is added a solution of 2,6-dichlorophenol-indophenol (containing 34.4 mg. in 1 liter of water).

In 1942 Loeffler and Ponting (*30*) used dilute metaphosphoric acid in fresh, frozen, and dehydrated fruits and vegetables in the determination of ascorbic acid.

A year later Ponting (*44*) made a special study of the loss of ascorbic acid in 13 acids, and came to the conclusion that only oxalic and metaphosphoric acids were suitable for extracting ascorbic acid from such vegetable materials as cabbage, broccoli, lima beans, peas, and strawberries, some of them frozen, using a Waring Blendor. A concentration of 1% for metaphosphoric acid was found to be satisfactory, using a ratio of 7 volumes of this dilute acid to 1 part of plant material, or higher.

In 1943 Horvath (*23*) treated 2-cc. portions of citrated beef plasma, diluted to 10 cc., with 0.1, 0.2, and 0.3 cc. of a 10% solution of sodium phosphate glass of pH 6.5 (Calgon), and acidified by hydrochloric acid (4%) to a pH of 3.8, which, as well as lower pH values, was found to be suitable for the precipitation of serum proteins. Complete deproteinization was observed where 0.2- and 0.3-cc. portions of the phosphate solutions were used. Deproteinization by acid phosphate glass (mole ratio Na_2O/P_2O_5 = 0.7152; pH of a 1% solution = 2.2), as well as by sodium trimetaphosphate and by sodium tripolyphosphate at a pH of 3.8 was also reported. Further studies were made on the solubility of the protein phosphate compound in neutral salts of mono-, di-, and trivalent metals, as well as in urea. The 1940 work of Briggs (*4*) explains the solubility of protein phosphate in neutral salts by its having many characteristics of a complex coacervate. Briggs found the titration curve of the protein-metaphosphate to be reversible, and confirmed the earlier findings of Perlmann and Herrmann (*40*) that no denaturation of the protein occurs through the action of metaphosphate.

In the same year Doan and Josephson (*10*), while studying the ascorbic acid content of evaporated milk, found that in milk heated to 240° F. for 15 minutes there appear reducing substances which react with the indophenol dye. The interfering substances

were successfully precipitated with the proteins by treating 25 ml. of the reconstituted milk with 26.7 ml. of coagulant consisting of 6% trichloroacetic acid and 4% metaphosphoric acid (the additional 1.7 ml. being used in order to compensate for the volume of the curd). The coagulum was immediately filtered and 10 ml. of the serum (equivalent to 10 ml. of the reconstituted milk) were treated with the indophenol dye.

In 1944 metaphosphoric acid was recommended by the Association of Official Agricultural Chemists (*1*) as a deproteinizing agent for the determination of ascorbic acid. The same year Gawron and Berg (*17*) observed that ferrous iron reduces dichlorophenol-indophenol in the presence of metaphosphoric acid, thus providing a basis for the stepwise determination of vitamin C and ferrous iron in the same aliquot.

In 1945 Kramer (*26*) used metaphosphoric acid in the determination of ascorbic acid content of some army foods. Fresh samples were covered with dilute metaphosphoric acid (3%), macerated in a Waring Blendor, and the vitamin C determined promptly in the filtrates. Vavich, Stern, and Guerrant (*48*), using a similar technique, found a final concentration of 3% metaphosphoric acid to be the optimum for blending unwashed raw peas, preserving the ascorbic acid in the presence of air for one hour, presumably by inactivating the enzymes.

In the same year, a U. S. patent was granted to Gordon (*18*) on the recovery of protein from milk whey or blood serum, where the first step consists in precipitation of the protein by means of metaphosphoric acid, at a pH range of 3.5 to 1.8.

Another U. S. patent, issued to Rushton (*45*), deals with a process for separating protein from aqueous industrial waste material by mixing with it a finely divided solid calcium metaphosphate (300-mesh) and maintaining the mixture in a slightly acid condition (generally in the order of pH 5), and digesting the mixture for a time sufficient to precipitate a substantial proportion of the protein in the form of a protein–phosphate complex, which is said to contain usually about 5% phosphorus pentoxide and 16% nitrogen. The patentee claims his method to be suitable in particular for the removal of protein from milk whey in order to recover subsequently milk sugar or lactic acid.

In 1945, Vavich, Dutcher, Guerrant, and Bechdel (*47*) used metaphosphoric acid in their study of the utilization and excretion of ingested ascorbic acid by dairy cows. Their technique was as follows:

Blood Plasma. Five milliliters of plasma and 10 ml. of 10% metaphosphoric acid were pipetted into a centrifuge tube containing 5 ml. of distilled water. The tube was centrifuged in order to facilitate a sharper separation of the precipitated proteins, and aliquots of the supernatant metaphosphoric acid extract were taken for ascorbic acid determinations.

Milk. An aliquot of milk was pipetted into an equal volume of 10% metaphosphoric acid solution. After shaking to break up aggregates of precipitated protein the mixture was titrated directly with indophenol dye solution to a pink end point which persisted for 30 seconds.

Rumen Fluid. The fluid was deproteinized by a 10% solution of metaphosphoric acid, and the ascorbic acid in the filtrate determined photoelectrically by a variation of the colorimetric method in which the unreacted dye was extracted with xylene.

The application of the indophenol–xylene extraction method to the determination of ascorbic acid in tomatoes and tomato juice was studied by Nelson and Somers (*37*), and preference given to 3% metaphosphoric acid as compared with a 5% sulfuric–2% metaphosphoric acid mixture because ascorbic acid is more stable in 3% metaphosphoric acid, and it is much easier to obtain perfectly clear filtrates using metaphosphoric acid alone than the acid mixture.

CONCLUSIONS

It is evident that deproteinization by means of metaphosphoric acid is already being used for a great variety of purposes. It seems to be generally accepted as the most reliable deproteinizing agent for the determination of ascorbic acid in plant and animal materials alike, mainly because of its stabilizing effect on the ascorbic acid and noninterference with the dichlorophenol-indophenol color reaction. Metaphosphoric acid has found favor in the determination of lactose in milk. It is also of value for deproteinization of clinical specimens, since it permits the determination in the filtrates of most of the constituents of interest to the clinician, giving reliable yields of the constituents without interfering with the standard colorimetric procedures.

The field of application of metaphosphoric acid in analytical chemistry is, however, not limited to deproteinization purposes. It can be used with equal advantage for the isolation of protein from agricultural and other products and the subsequent quantitative determination of the isolated protein. The analyst will undoubtedly find many new applications for the protein-precipitating property of metaphosphoric acid, and develop for it a number of new techniques.

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Application of Platinum Resistance Thermometry to Some Industrial Physicochemical Problems

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Platinum resistance thermometry is discussed in some detail, with mention of new techniques for construction of these thermometers and their convenient size. An automatic recorder facilitates the use of these thermometers. The cryoscopic method of determining the purity of a compound using platinum resistance thermometers is presented, together with the assumptions and limitations of the method. A modified ebulliometer fitted with a platinum resistance thermometer is introduced. A new type of boiling range apparatus incorporating a platinum resistance thermometer is presented and compared with the standard A.S.T.M. method. The increased accuracy resulting from the use of platinum resistance thermometers as well as the automatic recording feature fits these three applications well for industrial use.

THE reasons for the slight use of platinum resistance thermometers in industrial laboratories may be reviewed to advantage. Platinum resistance thermometers have been on the market for some time, but their high cost, calibration, fragility, size, and accompanying electrical measuring equipment have deterred many from using them. It is well, however, periodically to reexamine one's research tools in the light of more recent advances. Since the international temperature scale from −190° to 660° C. is defined in terms of a platinum resistance thermometer (*3*, *28*), it seems appropriate for precision measurements of such physical quantities as freezing point, boiling point, boiling range, vapor pressure, and the like to be made with a platinum resistance thermometer. Recent developments in equipment design, as well as demands for greater accuracy, have brought this admirable method of precise temperature measurement to a point of development such that it deserves to be more widely applied.

PLATINUM RESISTANCE THERMOMETRY

In 1871, Siemens (*18*) suggested that resistances be used as a means of measuring temperature. His materials of construction were faulty, causing many to discredit resistance thermometry. Some years later the monumental work of Callendar (*4*) laid the foundation for the reliability and precision of platinum thermometry as we know it today. In the 50-odd years since, some of those who have labored to perfect the platinum resistance thermometer are Sligh (*22*), Meyers (*12*), Mueller (*13*, *14*), Keesom (*9*, *10*), and Van Dusen (*26*).

The author has employed the methods of Meyers (*12*) for making resistance thermometers.

No. 38 B. and S. wire (approximately 0.1 mm., 0.004 inch, in diameter) of the highest purity platinum obtainable is wound on an iron piano wire No. 00 (approximately 0.2 mm., 0.008 inch, in diameter), so that between turns there is a free space equal to the diameter of the platinum wire. The iron wire is then dissolved out by hydrochloric acid, leaving a helix approximately 0.4 mm. (0.016 inch) in diameter. This platinum helix is wound on a notched mica framework in the form of a cross (see illustration in Meyers' paper). Two leads of pure gold wire size 32 B. and S. (approximately 0.2 mm., 0.008 inch, in diameter) are welded to each end of the coiled platinum helix with a small oxy-gas flame and threaded through the mica. (Where the thermometer is to be read to 0.01° C. this procedure is permissible, but if read to 0.001° a short piece of platinum wire is interposed between the helix and the gold lead to serve as a thermal dam for any possible heat being conducted along the gold leads.)

The four gold leads are kept insulated by mica disks 5 mm. in diameter (spaced by a short length of glass tubing) with four equidistant holes through which the wires pass. The coiled helix, having approximately 25 ohms' resistance at 0° C., occupies a space about 5 mm. in diameter by 20 mm. long. The temperature-sensitive element is thus concentrated within a fairly small space.

The whole assembly is placed in a Pyrex tube of 7-mm. outside diameter. The upper end of each gold lead is welded to a 1-cm. piece of 0.020-inch tungsten wire which is thermally platinized at each end.

Welding gold or platinum wires is facilitated by previously "tinning" the tungsten wire with platinum. A length of the tungsten wire is supported horizontally in a vacuum chamber (bell jar). Strips of 0.001-inch platinum foil 2 × 10 mm. are hung on the wire in the position where the tinning is desired. The bell jar is put in position, the air pumped out to a pressure of 1 micron or less, and an electric current sent through the wire. As the wire glows the oxides of tungsten vaporize and the platinum melts and "wets" the tungsten wire evenly in a homogeneous sleeve.

To the opposite end of the tungsten is welded a short piece of No. 28 B. and S. platinum wire, and the tungsten leads are sealed through the Pyrex wall. The thermometer is then heated to 400° C. for 8 hours, during which time it is continuously evacuated and refilled with dry air every half hour. After sealing off the gas filling neck (located adjacent to the tungsten lead seals at the upper end) with about 0.75 atmosphere of dry air in the thermometer, the four-lead copper cable is soldered to the platinum leads and the protecting head or handle is fastened in place with plaster of Paris. The platinum coil is then annealed by connecting it with a Variac and applying 55 volts across the 25-ohm coil for 40 seconds. This heats the platinum to redness and relieves any strains due to winding, etc., to ensure permanence of calibration. The annealing process is continued as follows:

50 volts for 2 minutes
45 volts for 6 minutes
40 volts for 10 minutes
35 volts for 20 minutes
30 volts for 30 minutes

Figure 1 shows the size of the finished thermometers, which in dimensions and utility compares very favorably with the usual mercury thermometer. It is convenient to construct the thermometer with a ground-glass joint for insertion into various pieces of apparatus.

Platinum metal is admirably suited to serve as the resistor material, because of the very regular and reproducible relation existing between the resistance and temperature.

This relation has often been fitted with a parabolic equation of the form

$$R_t = R_0 (1 + At + Bt^2) \qquad (1$$

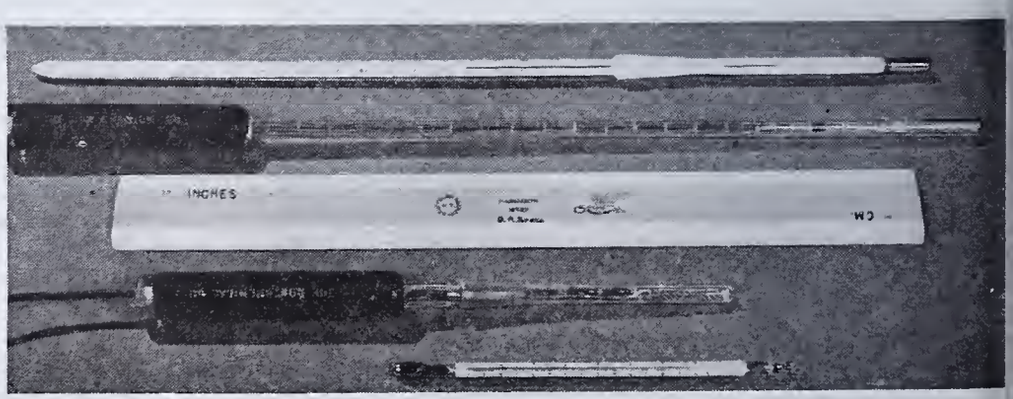


Figure 1. Comparison of Platinum Resistance Thermometers with Conventional Mercury Thermometers

here R_t is the resistance at temperature t, R_0 is the resistance at ° C., and A and B are constants. To avoid solving Equation 1, is sometimes put in the form

$$t = \left(\frac{R_t - R_0}{R_{100} - R_0}\right) 100 + \delta \frac{t}{(100 - 1)} \frac{t}{100} \qquad (2)$$

here R_t, R_0, and R_{100} are the measured resistances at $t°$, 0°, and 00°, respectively, and δ is a constant obtained by measuring the esistance at some other fixed temperature, usually the sulfur oiling point. Equation 2 will reproduce the temperature-resistnce relationship with sufficient accuracy from −40° to 660° C., ut at low temperatures does not reproduce the thermodynamic emperature scale with sufficient accuracy, so in 1925 Van Dusen 26) proposed the formula

$$= \left(\frac{R_t - R_0}{R_{100} - R_0}\right) 100 + \delta \left(\frac{t}{100} - 1\right) \frac{t}{100} + \beta \left(\frac{t}{100} - 1\right) \frac{t^3}{100^3} \quad (3)$$

here β is another empirical constant obtained by measuring the esistance at a fixed low temperature, usually the boiling point of f oxygen. The form of these equations can be appreciated best y a worker in this field. After constants δ and β are computed, ne resistance of each thermometer is calculated for each 10° inerval (see Table I).

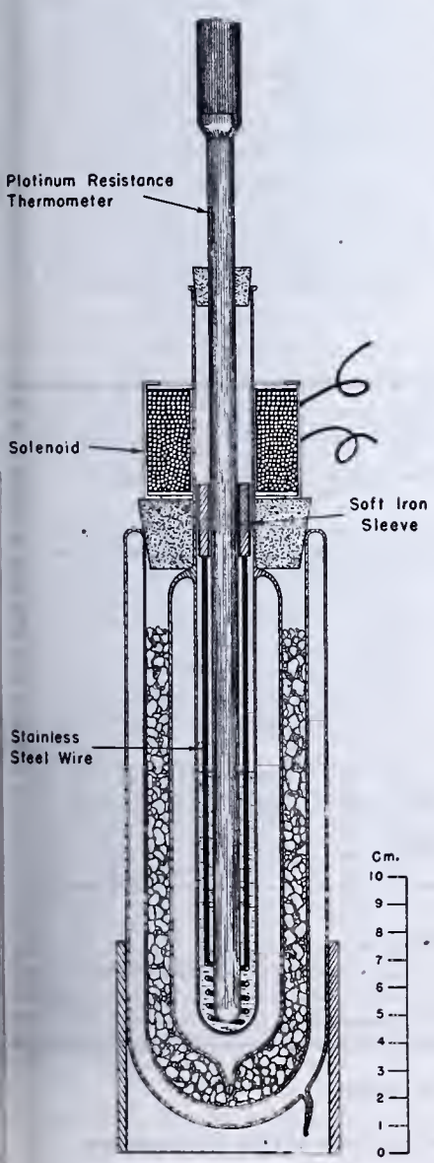
Table I. Platinum Resistance Thermometer, NBS 690

(R_0 = 25.540Ω. R_{100} = 35.560Ω. δ = 1.49$_5$. β = 0.110$_8$)

−T° C.	RΩ	ΔRΩ	T° C.	RΩ	ΔRΩ
0	25.540		0	25.540	
		1.018			1.016
10	24.522		10	26.556	
		1.022			1.012
20	23.500		20	27.568	
		1.025			1.009
30	22.475		30	28.577	
		1.028			1.007
40	21.447		40	29.584	
		1.031			1.003
50	20.416		50	30.587	
		1.036			1.001
60	19.380		60	31.588	
		1.039			0.997
70	18.341		70	32.585	
		1.043			0.995
80	17.298		80	33.580	
		1.048			0.992
90	16.250		90	34.572	
		1.052			0.988
100	15.198		100	35.560	

Inspection of Table I will disclose the fact that $\Delta R\Omega$ for each 10° interval is substantially 1 ohm, and that it changes slowly enough so that linear extrapolation of each 10° interval is permis-

igure 2 (Below). Freezing. 'oint Apparatus



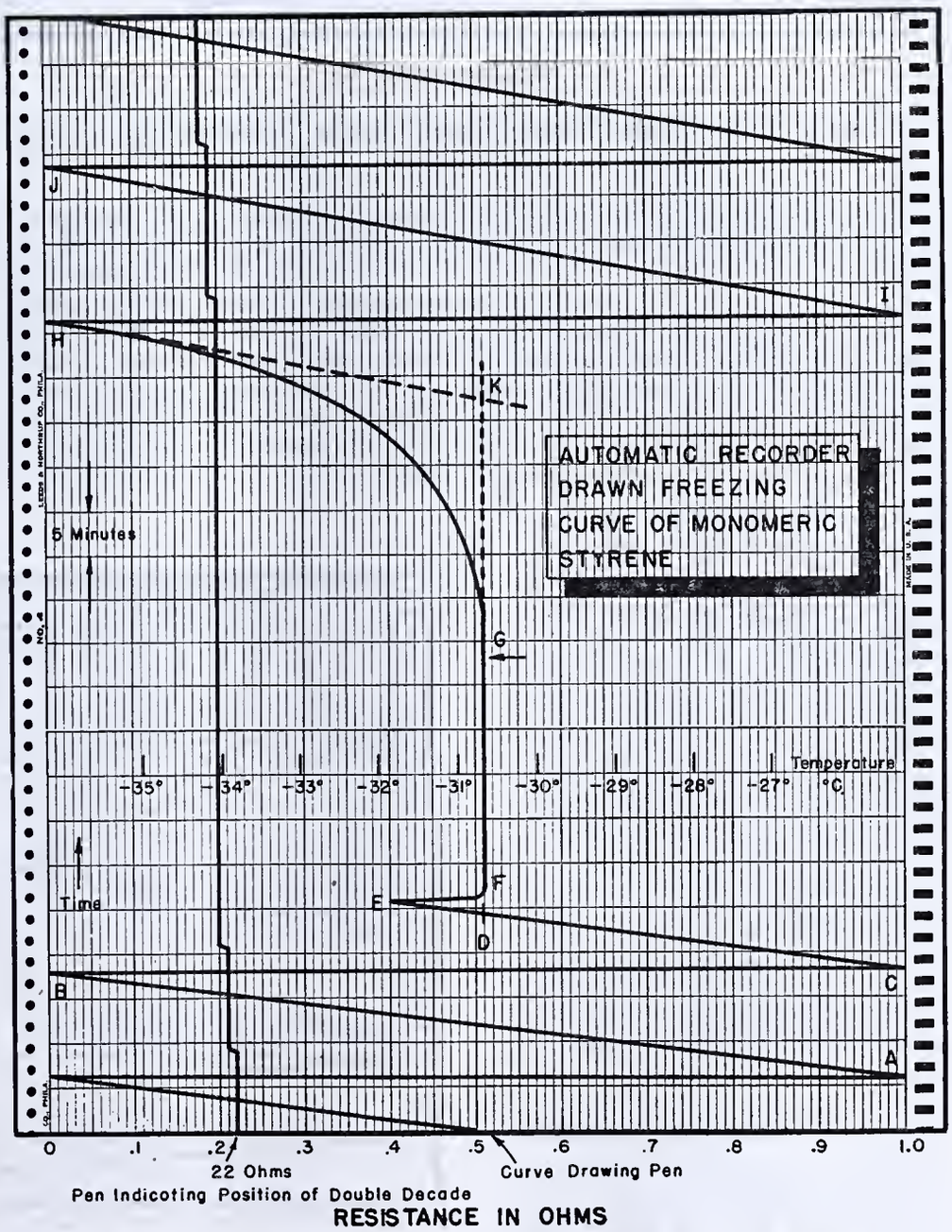


Figure 3. Automatic Recorder-Drawn Freezing Curve of Monomeric Styrene

Because of the necessary reduction of recorder-drawn figures, the original pen traces have been retouched to make them broader

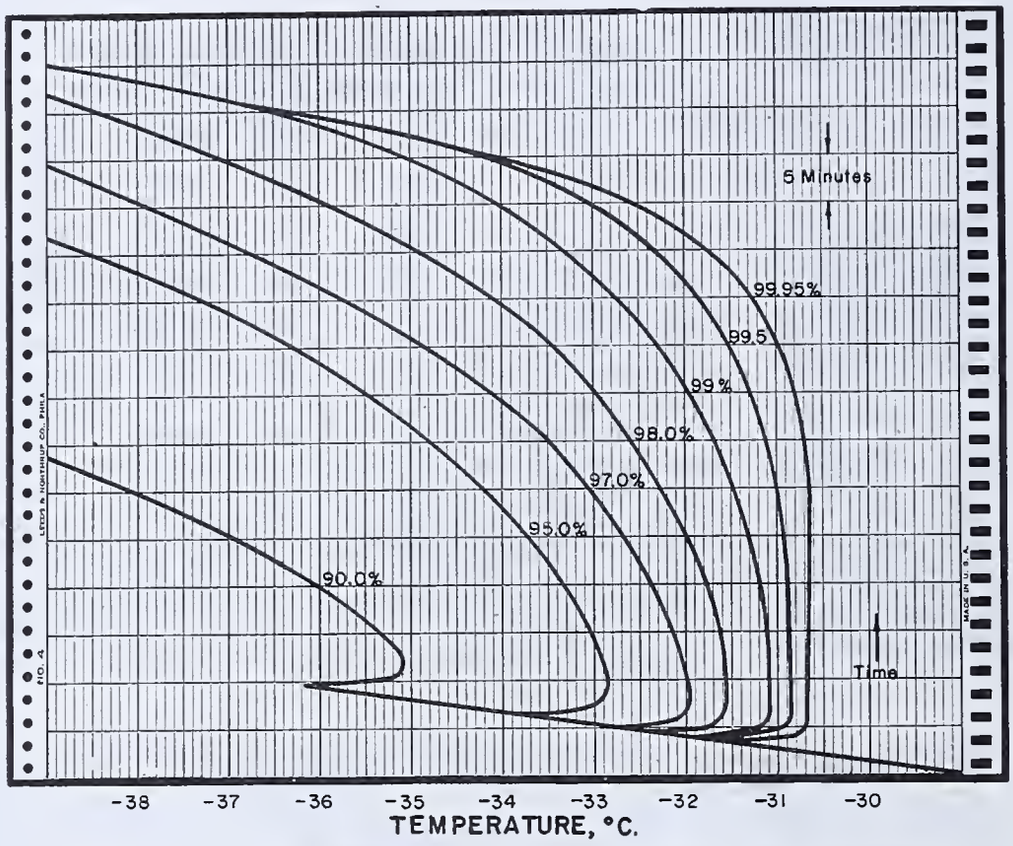


Figure 4. Superimposed Freezing Curves of Styrene Diluted with Ethylbenzene

the true freezing point is known, if th time-temperature curve is observe during the freezing process the tru freezing point can be calculated an the method is more cogent. It is true however, that the method rests o certain assumptions, and the result are proportional to the degree to whic the assumptions are fulfilled.

As outlined originally by White (*29*) and more recently by Mair, Glasgow and Rossini (*11*), the estimation of th amount of solid-insoluble, liquid soluble impurity from a time-tempera ture freezing curve involves the follow ing assumptions:

1. The impurity remains entirel in the liquid phase during the time tha the temperature lowering is being de termined.
2. The rate of crystallization re mains constant.
3. Complete thermal equilibriu exists between the solid and liqui phases.
4. That an ideal solution is forme between the liquid phase and the solid insoluble, liquid-soluble impurity, or, this system forms a nonideal solutio that it is of sufficient dilution that may be treated as ideal.

sible when only 0.01° C. accuracy is sought. This greatly facilitates the calculation of measured values of R to the corresponding temperature.

Since 1 ohm is equivalent to 10°, it is necessary to measure the electrical resistance of the platinum coil to within 0.001 ohm in order to calculate the temperature to 0.01° C. Resistance measurements of this order of magnitude have long been adequately made with hand-operated bridges (*13*), but are somewhat tiresome and time-consuming. To eliminate this toil, a completely automatic resistance recorder has been developed in this laboratory.

It consists essentially of a 25-cm. (10-inch), constant-speed, strip chart recorder with a full-scale range of exactly 1.000 ohm, but with the ability to shift this range automatically anywhere from 0 to 100 ohms as the occasion demands. A second pen simultaneously records the range in which the instrument is operating. One can read the chart to within about 0.01 inch, corresponding with a practical limit of ±0.01° C. in the precision of the instrument. A more complete description of this machine has appeared elsewhere (*24*).

As the remainder of this article demonstrates, the perfection and construction of an automatic recorder for a platinum resistance thermometer should considerably increase the usefulness and scope of that instrument in precision thermometry.

CRYOSCOPIC DETERMINATION OF PURITY

A number of workers (*1*, *8*, *11*, *17*, *19*, *20*, *21*, *23*, *29*) have pointed out the fact that, while the initial freezing point of a compound may be employed to calculate its purity when

Thus the method gets progressivel less effective as the concentration of the impurities increases, bu what is very important, the higher the purity, the more con pletely are the assumptions satisfied. Usually the method open to serious question if the sample is less than 90% pur At 95% the method is fairly reliable, but from 98% on up th results become progressively more excellent, depending, of cours on the accuracy to which the resistance is determined.

It is paramount that the sample freeze to a crystalline soli Glass formation is sometimes encountered, rendering the cry scopic purity determination impossible. Various techniques hav

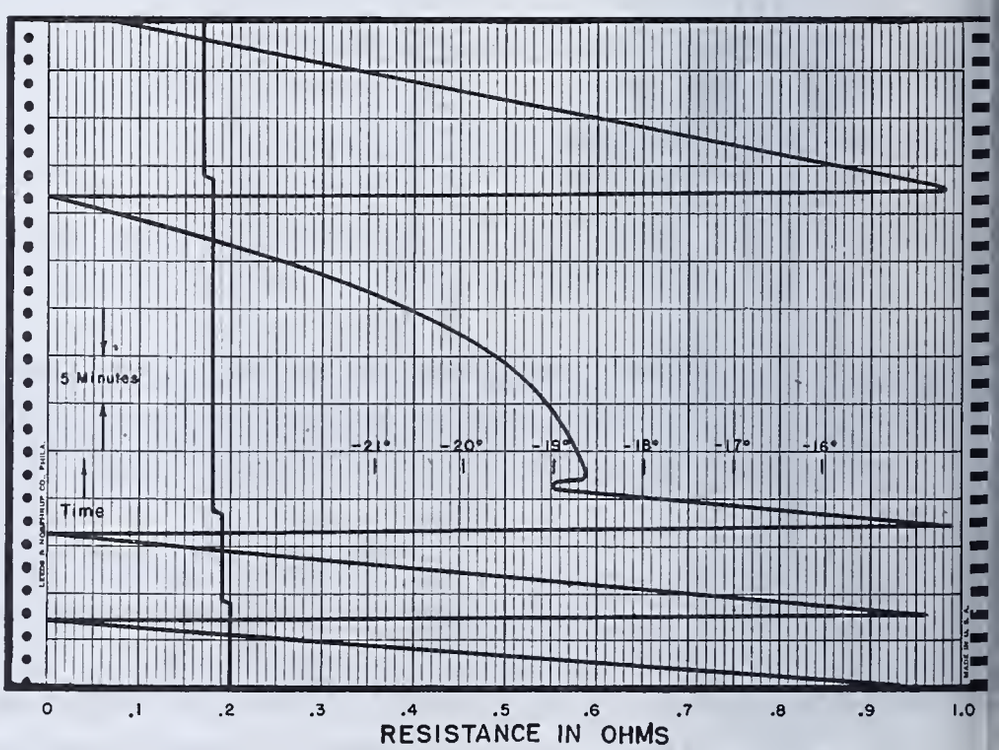


Figure 5. Automatic Recorder-Drawn Freezing Curve of Para-Chlorostyrene

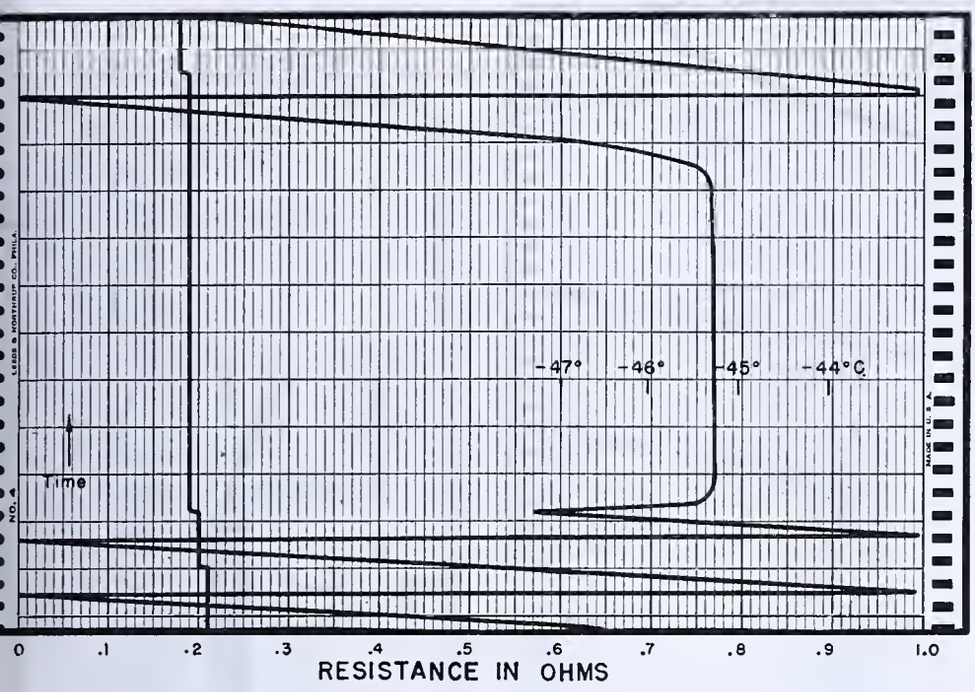


Figure 6. Automatic Recorder-Drawn Freezing Curve of Chlorobenzene

fact that the temperature of the refrigerant is always maintained essentially constant, the cooling rate is constant, thus satisfying the requirements of assumption 2. Several sample containers are available with different degrees of evacuation, permitting proper choice of container, so that near the freezing point the cooling rate will be in the range 0.8° to 1.0° C. per minute. Figure 3 is a time-temperature cooling curve of monomeric styrene automatically recorded by the machine referred to above.

The usual method of plotting time-temperature curves is to plot temperature as the ordinate increasing upward and time as abscissa increasing toward the right. The type of automatic recorder developed for this purpose makes use of a strip-chart machine which plots time increasing upward on the chart as the ordinate, with abscissa recording the resistance increasing

en used to induce crystallization, ıong which may be mentioned ɛding (provided some seed is avail-le), vigorous stirring, scratching the ıer wall of the sample tube, plung-ɛ into the liquid a wire chilled th liquid nitrogen, and the presence an inert crystal of the same lattice nensions.

A number of different cryoscopic ɛhniques have been devised and blished (*1*, *8*, *11*, *17*, *19*, *20*, *21*, , *29*); this list does not pretend be complete. Each has some special ɛrit which best fits it to do some rticular job. The procedure de-ɛibed here has been devised to general laboratory usage, and s been in use now for some 4 ars.

Fifteen milliliters of sample are troduced into a test tube having unsilvered vacuum jacket around lower portion (see Figure 2). Some equate means of stirring the sample a rate of 40 to 80 strokes per minute provided, such as a solenoid or a nverted windshield wiper reciprocat-ɛ stirrer.

The test tube is fitted with a rub-r stopper which serves to support e test tube in a wide-mouthed ermos bottle containing the re-gerant. Crushed dry ice in a mix-re of half and half chloroform and rbon tetrachloride (this mixture is nflammable) or liquid nitrogen is e refrigerant used, depending upon e degree of cooling required. Sup-ɛrted by a cork stopper at the top of e sample tube is the resistance ermometer.

Because of the poor heat transfer rough the vacuum jacket, and the

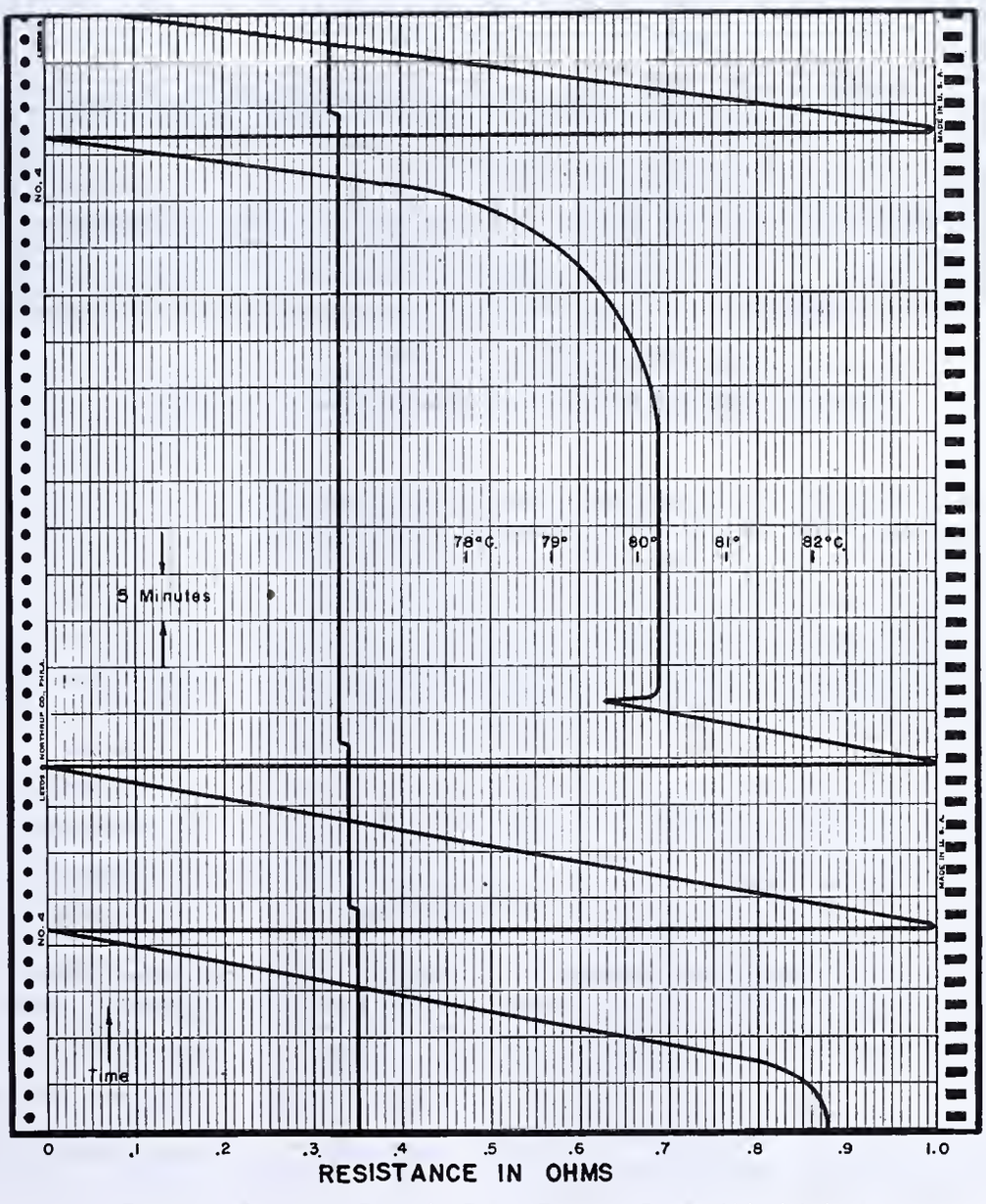


Figure 7. Automatic Recorder-Drawn Freezing Curve of Naphthalene

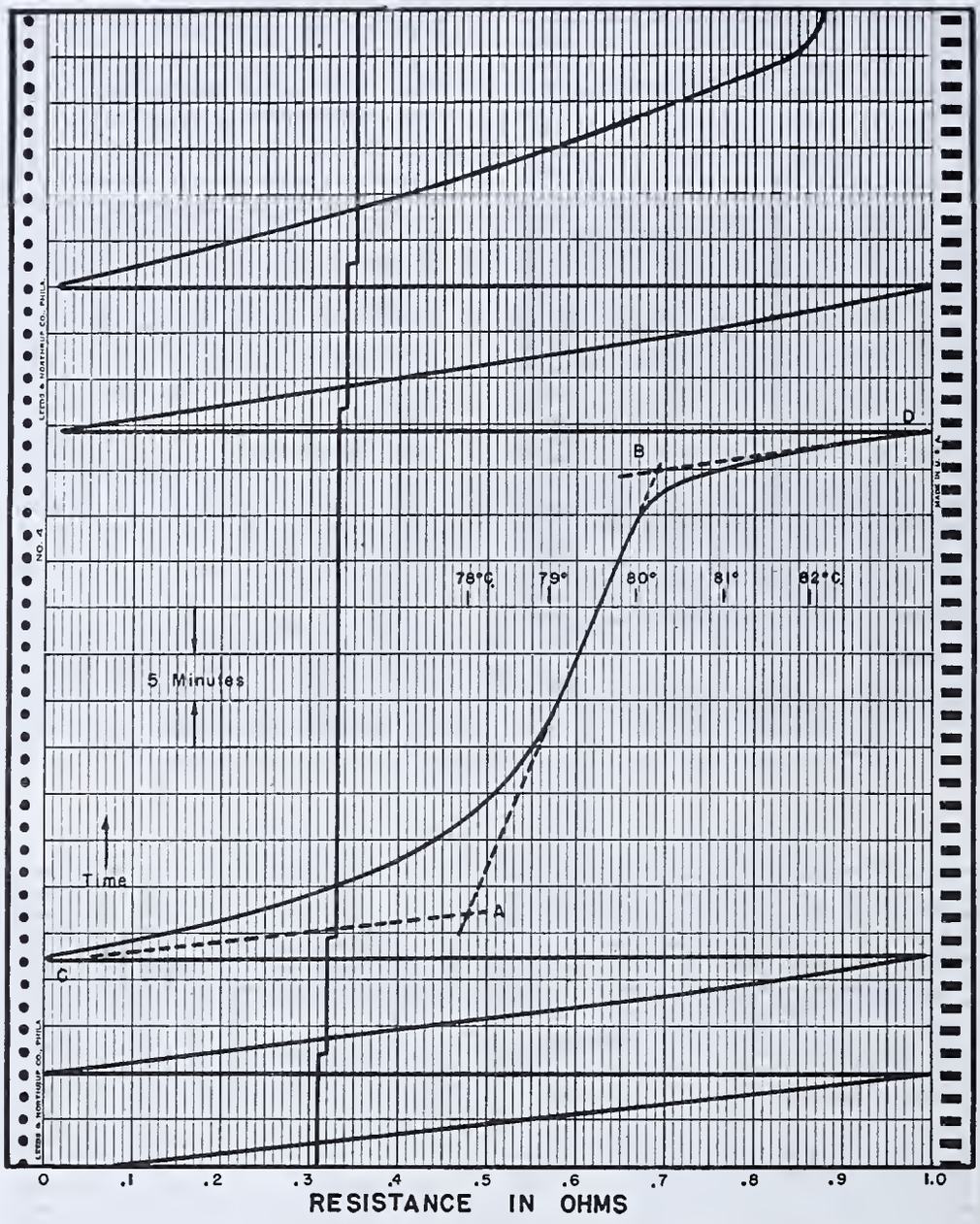


Figure 8. Automatic Recorder-Drawn Melting Curve of Naphthalene

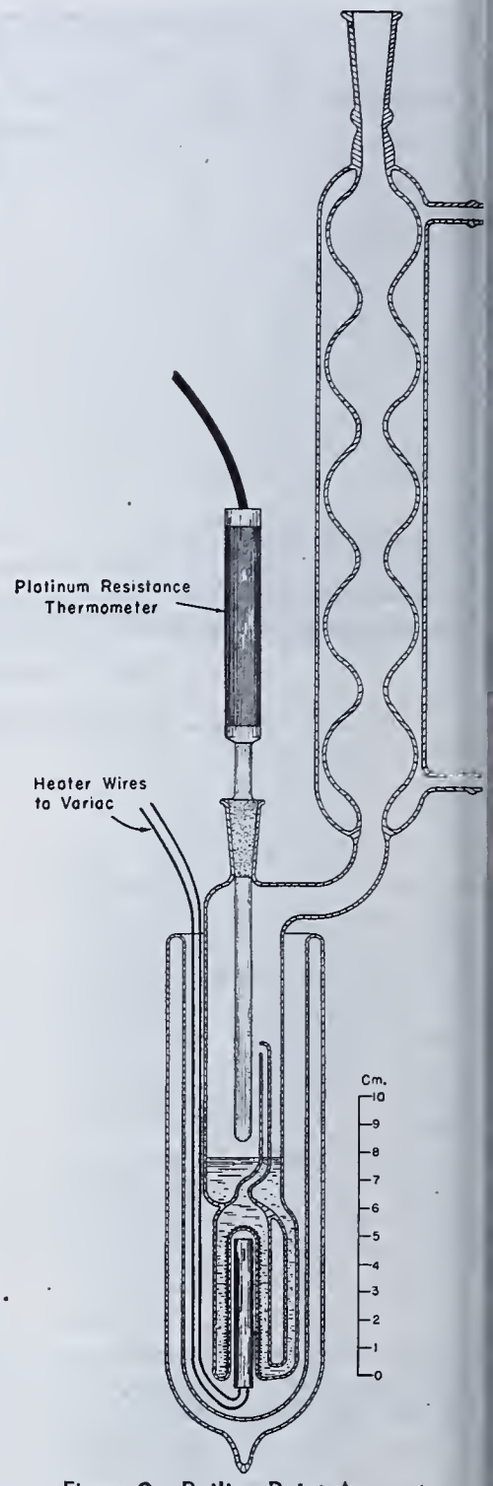


Figure 9. Boiling Point Apparatus

toward the right. The recorder has proved so useful that the users early learned to make the necessary mental geometric translation, and now find it just as convenient to work with as the older system.

The temperature of the sample decreases from A to B, then the range shifts, and the temperature of the sample decreases from C to E. At point E, crystallization is initiated and the temperature of the sample (which is supercooled at E) rises to F where the solid and liquid phases are in thermal equilibrium. The temperature follows from G to H (as the sample crystallizes) where the range again shifts and the sample continues to cool in the solid state from I to J. Sometime during the stirring, the stirrer freezes fast (the magnetic impulse is not strong enough to cause any damage, or if the windshield wiper is used, a flexible link is included) but this causes no trouble.

In working up the data, a line of constant temperature, F, is drawn from F to K, and JI is extrapolated back to K. Now the sample is all liquid at D and if it were 100% pure, it would have been completely frozen in the ideal case at K. Then the sample must have been half frozen at G, the midpoint between D and K. If the impurities concentrate in the mother liquor in accordance with assumption 1, when the sample is half frozen the impurities are twice their original concentration, and the difference in temperature ΔT between F and G is due to this doubling of the impurity concentration. Then pure 100% material would freeze at a temperature ΔT higher than the temperature F, and thus we have a method of determining the freezing point of the pure material from a sample containing impurities.

To a first approximation (neglecting higher terms whose maximum contribution is 2% or less), the purity of the sample is related to ΔT by the following relationship:

$$X = A\ \Delta T \qquad (4$$

where

X = mole fraction of impurity

A = cryoscopic constant = $\frac{\Delta H_f}{RT_f^2}$

ΔH_f = molar latent heat of fusion of material in calories

T_f = absolute temperature of freezing point

R = gas constant per mole = 1.987 calories

In the case of Figure 3 $\Delta T = 0.02°$ C. (hardly observable i the reduced chart) and the initial freezing point of 100.00% pur monomeric styrene should be $-30.63°$ C. Also since ΔT = $0.02° \pm 0.01°$ C., our sample contains 0.05 ± 0.02 mole % im purity and thus has a purity of 99.95 ± 0.02 mole %.

Figure 4 shows what happens to the freezing curve as monc meric styrene is diluted with small amounts of ethylbenzene The initial freezing temperature drops, but even more sensitive i the length of the plateau or straight portion of the coolin curve.

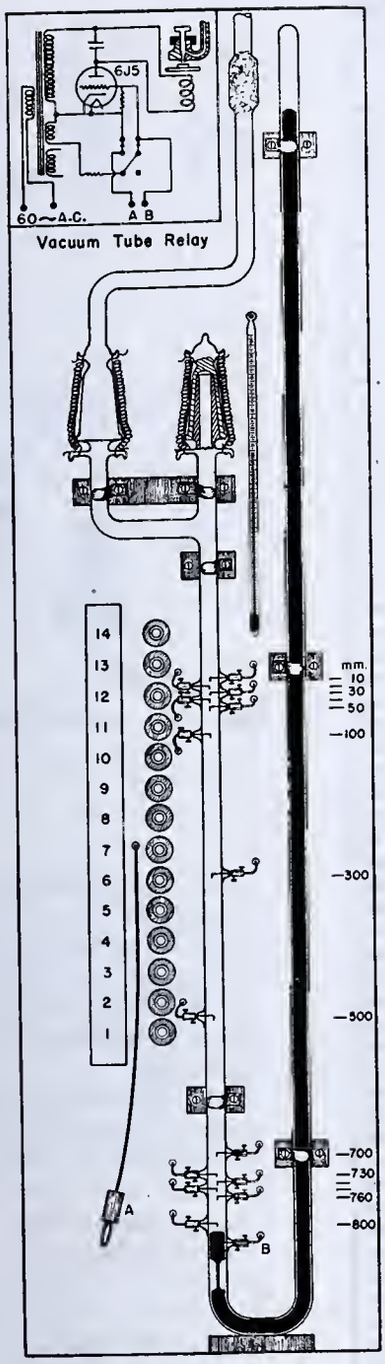


Figure 10. Contacting Barometer

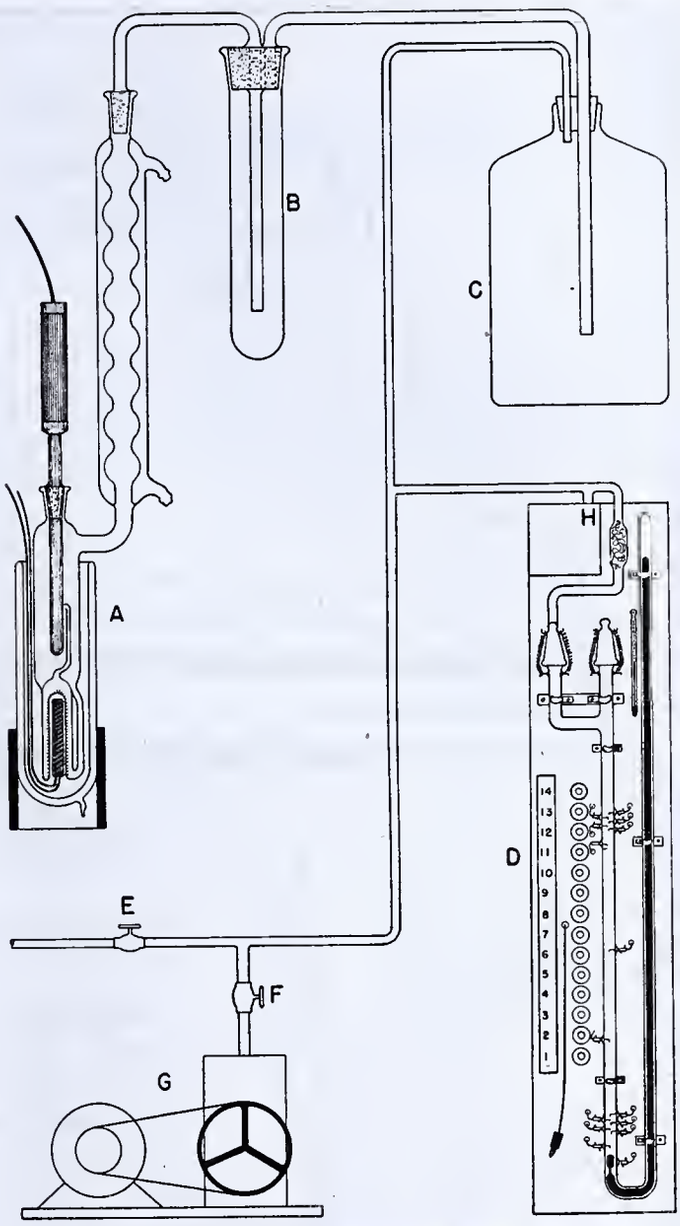


Figure 11. Constant-Pressure Boiling Point Apparatus

Figure 5 shows the freezing curve of a sample of *p*-chlorostyrene which turned out to be about 85 mole % pure. In the light of Figure 4, an experienced operator can tell at once the approximate purity by looking at the shape of the curve and the plateau. The nearest freezing curve to ideality that has been encountered is shown in Figure 6, and probably represents some of the purest chlorobenzene that has been prepared. Figure 7 shows a freezing curve of naphthalene, and is given here in order to compare it with the melting curve shown in Figure 8.

Because the thermal conductivity of organic solids is much less than the liquid, the melting curve does not present as good conditions for realizing thermal equilibrium of the melt. Hence, while the melting point can be determined accurately and checks the initial freezing point very well, measurements of ΔT at a half melted point are often in error. Nevertheless, occasions arise, as, for example, when the sample has supercooled some 20° or 30° before crystallization starts, and so much heat has been lost that the sample never reaches a thermal equilibrium between solid and liquid state, when the initial freezing point is known to be in error. It is then a very convenient thing to let the recorder draw the melting curve (it is necessary to melt the sample to retrieve the resistance thermometer) and thus gain more information about the sample.

Referring to Figure 8, as the sample melts, it attains a thermal melting equilibrium indicated by the dotted line, *AB*. The solid state line is extrapolated at *CA* and the liquid state at *DB*. Point *B* is the melting point of the compound which can be compared with the freezing point obtained in Figure 7 (actually this material was melted in a small furnace as shown in Figure 8, then cooled and the freezing curve in Figure 7 obtained). This sample of naphthalene was about 99.95 mole % pure.

Over the last 4 years, some 125 different organic compounds have been frozen in this laboratory. Frequently in the case of isomeric mixtures, the freezing point has been one of the most reliable analytical tools available. The list includes plastic monomers, hydrocarbons, alcohols, amines, halogenated hydrocarbons, sulfur compounds, acids, aldehydes, ketones, esters, etc., and the freezing temperatures range from −160° to +200° C. This work has served to stress the paucity of heat of fusion data needed to calculate the cryoscopic constant.

EBULLIOMETRY OF LIQUIDS

The problem of accurately measuring the temperature of a liquid boiling under precisely known pressures has received con-

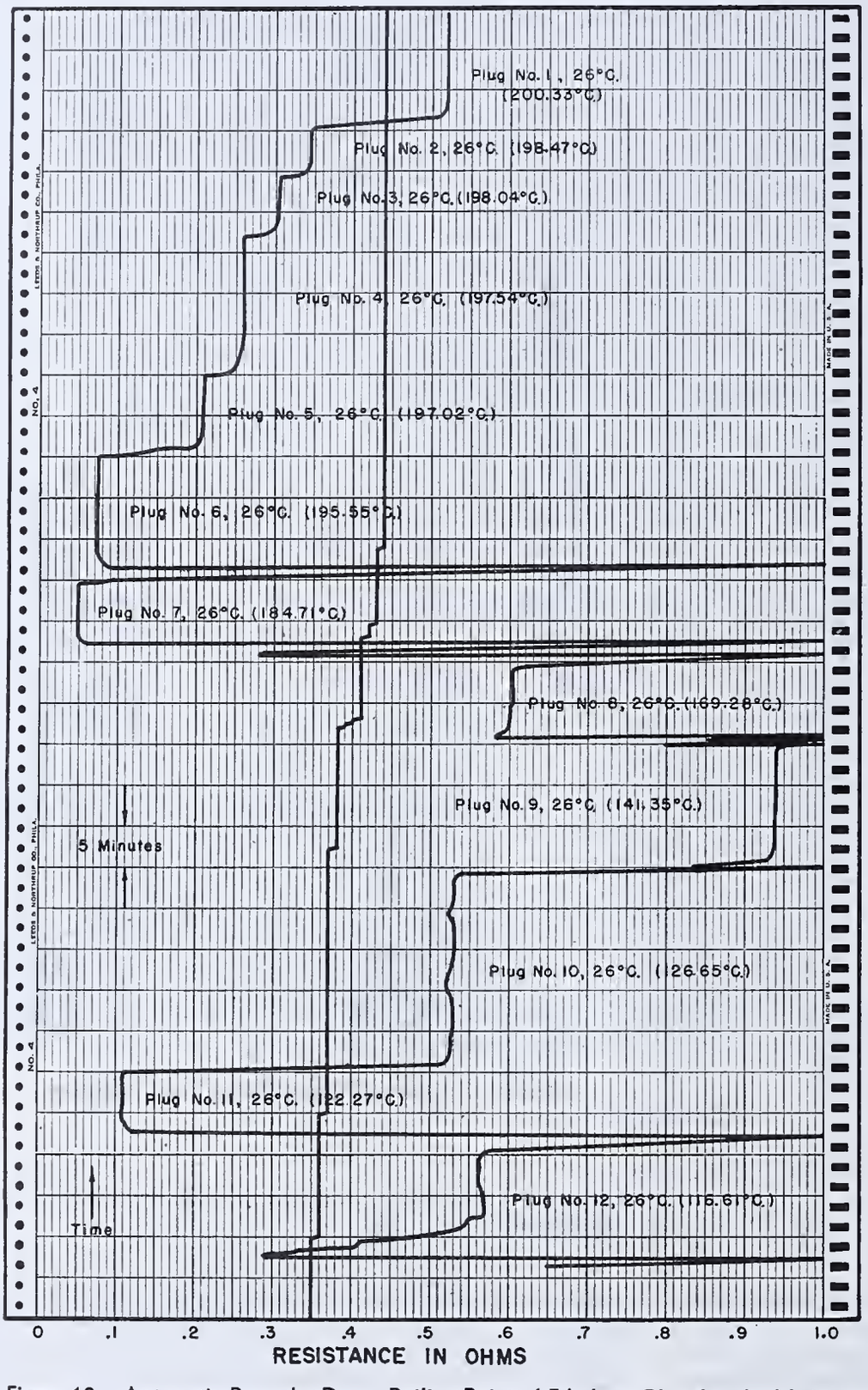


Figure 12. Automatic Recorder-Drawn Boiling Point of Ethylene Glycol under Various Pressures

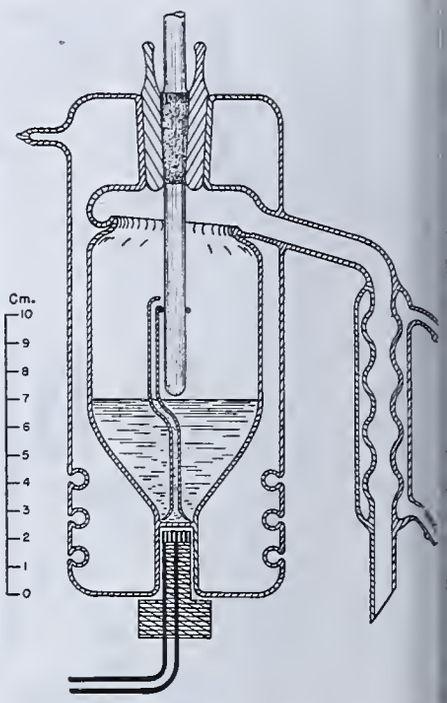


Figure 13. Boiling Range Apparatu

siderable attention (*5*, *6*, *7*, *16*, *25*, *27*, *30*, *31*). Over the last few years a boiling point apparatus which combines accuracy and convenience has been developed in this laboratory. It consists of a boiler and condenser, a special barometer for measuring the pressure, and auxiliary pressure control apparatus.

Figure 9 is a diagram of the boiler, which consists of a small reservoir with a re-entrant well in the bottom. The re-entrant well contains an electric cartridge-type heater (Chromolox C201), and has powdered Pyrex fused to its inner wall to promote smooth boiling. The liquid boils and the vapor pushes hot liquid before it up the Cottrell pump where together they spew out on the platinum resistance thermometer. The vapor is condensed and the liuqid returns to the bottom of the reservoir where it is recycled. To prevent heat loss, the boile is mounted inside a thermos bottl Rapidity of recycle prevents supe heating, and the temperature recorde by the resistance thermometer soc comes to a thermal equilibrium (s Figure 12).

The pressures in this apparatus a measured by a contacting barometer Rossini and co-workers (*31*) seale tungsten wires into the permaner vacuum side of their barometer, ar arranged it to control the pressure c the system. With this scheme, tl authors have found some slight g leakage over a period of several montl around the tungsten wires. A ne model built with the contacts on tl open side of the barometer seems t work much better.

Figure 10 shows a diagram of tl contacting barometer, which is mac of 10-mm. Pyrex tubing with 20-m tungsten wire sealed into the ope arm. By means of a set of bindir posts a plug can easily connect any the contact points with a vacuum tub relay. The armature of the relay is fitted with a rubber pad whic can cover or uncover a small hole whose free opening is adjus able by a small needle valve, *H* (Figure 11).

Figure 11 shows the pressure control apparatus. When it plugged into a pressure less than atmospheric, the vacuum pum *G*, continually exhausts the system at a rate governed by *I* When the mercury rises to the appropriate tungsten conta point in the contacting barometer, *D*, the air leak, *H*, opens an admits air until the mercury falls below the contact, thus shuttin off the air leak. By careful adjustment of valves *F* and *H* tl mercury column (and indirectly the pressure) can be controlle to about ±0.1 mm. on either side of the contact point. Thes fluctuations in pressure are smoothed out by a 5-gallon surg

nk, *C*, between barostat *D* and iler *A*, so that the pressure fluctuaon on the boiler (as determined by independent method) is about ±0.02 m. *B* is a dry ice trap to prevent pors from the sample from invading e rest of the system. When plugged to a pressure greater than atmosheric, compressed air is fed in at *E*, d *F* is closed. The operation is the me, except that the normal position the vacuum tube relay is now reersed, because the air leaks out inead of in. By placing pure distilled ater in the boiler and measuring the iling point of the water with a atinum resistance thermometer for ch contact point, the pressure value each contact point was interpolated om the accurate water vapor pressure ta of Osborne and Meyers (*15*).

This scheme eliminates considerans of pressure drop between the ints where the temperature and essure are measured. Because the rometer also acts like a thermomer it must always be corrected to C.

Thus by adjustment of the appatus, the boiling point at some 14 essure points (see Figure 12) can be easured to ±0.02 mm. of mercury. xamination of Figure 12 will disclose e fact that at lower pressures (plugs) and 12) there is difficulty in esblishing a steady temperature equilibum. This difficulty is encountered nerally in any dynamic boiling point ethod.

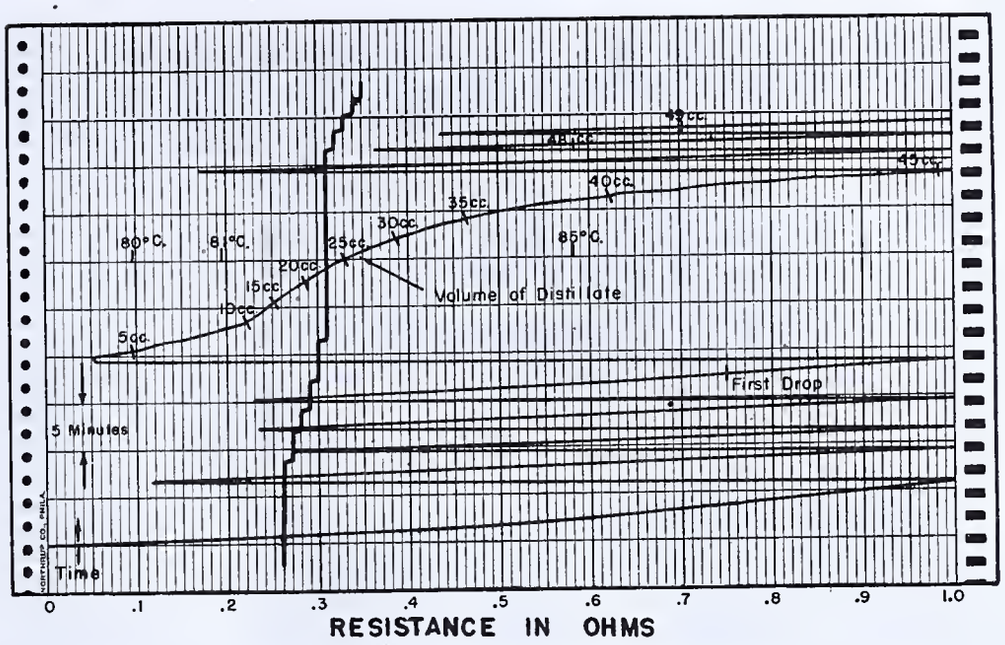


Figure 14. Automatic Recorder-Drawn Boiling Range of Unrefined Benzene

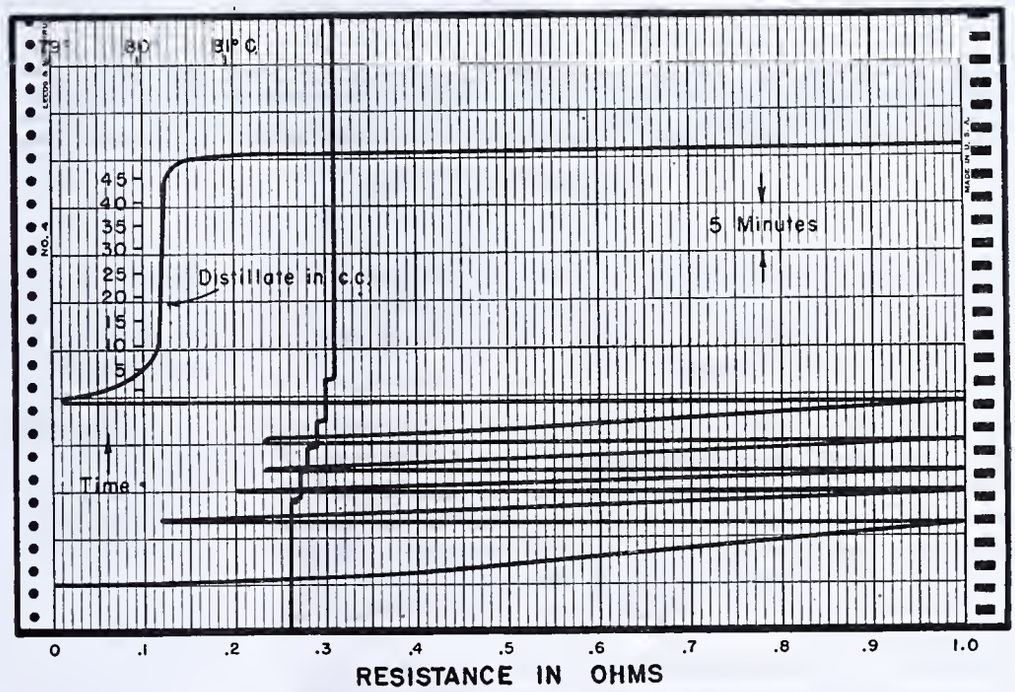


Figure 15. Automatic Recorder-Drawn Boiling Range of A.C.S. Reagent Grade Benzene

BOILING RANGE OF A LIQUID

Much time is spent in determining ie boiling range of a particular liquid order to get some idea of its purity. tandard prescribed methods for acmplishing this have been in use for number of years (*2*). It would seem iat this is an instance in which an itomatic recording resistance therometer could serve to advantage.

Figure 13 gives a diagrammatic sketch of an apparatus degned to be used to determine the boiling range of a liquid.

Fifty milliliters of liquid are placed in the central chamber, and ie Cottrell pumping tube is inserted and held in place by the sistance thermometer. The liquid is boiled by means of a eater, which is a flat pancake-type coil of Nichrome ribbon on ie upper end of an asbestos plug, and which is inserted in the ottom heater well. The whole apparatus is surrounded by a nventional vacuum jacket. Once the heat is turned on, it is ept at a constant value, so that the boiling rate will always be nstant and comparable. The trough at the top of the inner hamber prevents vapor from condensing and running back, once

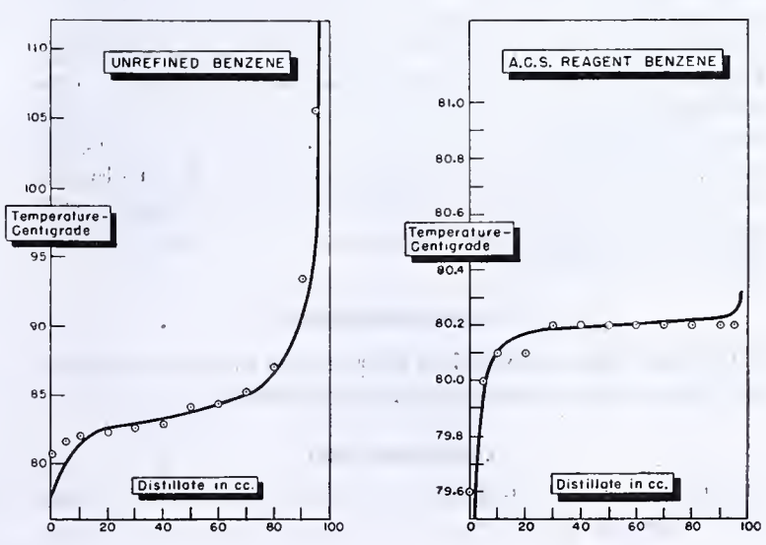


Figure 16. Comparison of This Boiling Range Method with the Standard A.S.T.M. Boiling Range Method

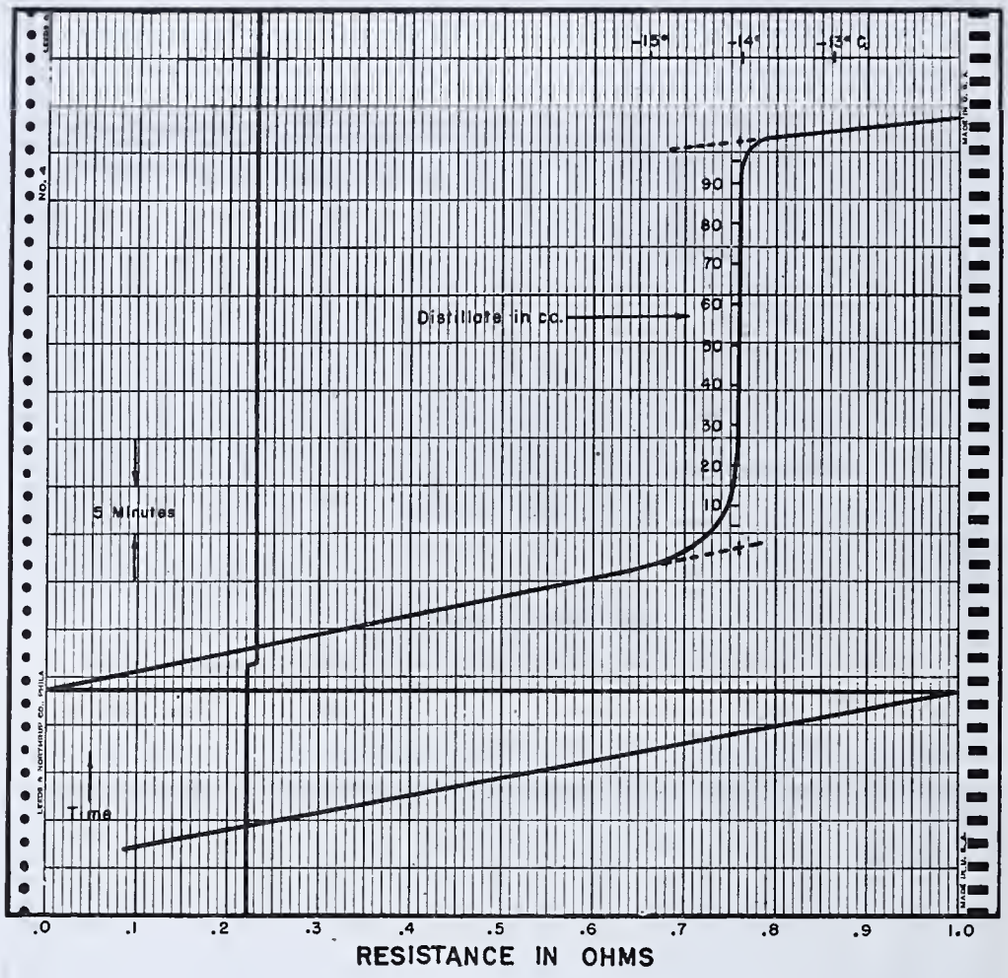


Figure 17. Automatic Recorder-Drawn Boiling Range of Vinyl Chloride

boiling has started. The vapors are condensed and recovered in a graduated cylinder. Once boiling starts, liquid and vapor are pumped over the resistance thermometer which is thus in thermal equilibrium with each component.

Figure 14 shows the time-resistance record of the boiling range of a sample of unrefined benzene, while Figure 15 shows the automatically traced time-resistance record of the boiling range of a sample of A.C.S. reagent grade benzene. In Figures 14 and 15 the distillate was caught in a graduated cylinder and the record kept on the chart. Both samples were also run according to the standard A.S.T.M. procedure (*2*) and Figure 16 shows the data replotted for comparison (the milliliter values for the 50-ml. samples were doubled).

Obviously the recording thermometer working every second can detect temperature changes more quickly than an operator. This is an important factor, particularly on the ends of the curves. Because the rate of heat input is constant, it is not necessary to observe each unit of 5 or 10 ml. of distillate as it comes over, but the recorder chart can be divided into 10 or 20 equal parts with a ruler as shown in Figure 17 and the temperatures read off at once. Figure 17 is a record of the boiling range of vinyl chloride. The temperatures marked on the chart indicate the narrow boiling range of this material. This apparatus embodies a rapid control method to determine the purity of this product. By making the boiling range determination automatic in so far as possible, the operator can measure more boiling ranges in the same time with little or no extra effort.

ACKNOWLEDGMENT

The author wishes to express his gratitude to The Dow Chemical Company for permission to publish this effort.

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Volumetric Quantitative Determination of —SOONa Group in Sodium Benzene Sulfinate

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THE quantitative precipitation of aryl sulfinic acids as ferric salts, reported in 1909 by Thomas (*3*), has been employed in a variety of procedures as the principal reaction in the quantitative determination of the sulfinic acids. Thomas mentioned the formation of sulfonyl chlorides, by reaction with sodium hypochlorite in sodium carbonate solution, but apparently made no attempt to use this reaction as a quantitative tool.

Krishna and Singh (*2*) reported using the ferric salt reaction, employing a solution of ferric chloride of predetermined concentration, filtering off the ferric salt, and determining the excess of ferric chloride. Until recently, Thomas' ferric salt reaction in very concentrated solution has been employed in this laboratory, for want of a better method.

The approximate equivalent of 2.5 grams of sodium benzene sulfinate is dissolved in 25 ml. of distilled water, and made acid to Congo red by dropwise addition of concentrated hydrochloric acid. Fifteen milliliters of 15% ferric chloride solution are added and the resultant slurry is swirled for several minutes until the precipitate turns light yellow. The precipitate is filtered and washed 6 times with 5 ml. of water, and 3 times with 5 ml. of methanol, care being taken to fill in the cracks which form on the filter. The precipitate is then removed to a large crucible (5 cm.) and carefully ignited to Fe_2O_3.

There are disadvantages in using this procedure. The ferric salt is soluble in excess ferric chloride. The precipitate is so voluminous that it is very difficult and time-consuming to wash it free of salts, and the mass on the filter nearly always cracks. Sodium sulfite, known to be present in commercial sodium benzene sulfinate, interferes with the accuracy of the method. Comparative runs on equal samples have also shown that the weight of precipitate becomes less as the initial concentration of sodium benzene sulfinate is reduced.

These objections also apply to the Krishna and Singh method in its application to commercial production.

Krishna and Das (*1*) report a complex gas volumetric analysis involving the liberation of iodine from a mixture of potassium iodide and potassium iodate, and utilization of the free iodine to liberate oxygen from hydrogen peroxide. The success of this method, by admission of the authors, depends on the high purity of the reagents and also on an approximate adjustment of the proportions in which they are used. These prerequisites, in addition to the complexity of the procedure, seem to disqualify it as a simple method.

Thomas' reaction of sodium benzene sulfinate with sodium hypochlorite was investigated and found to give accurate and reproducible results. When sodium benzene sulfinate with only slight excess of sodium carbonate was treated with concentrated (12%) sodium hypochlorite, a small yield of sulfonyl chloride was obtained. However, under the conditions employed in the method described below, the reaction is probably

$$C_6H_5SO_2Na + NaOCl \longrightarrow C_6H_5SO_3Na + NaCl$$

Even if some sulfonyl chloride is formed, the equation

$$C_6H_5SO_2Na + NaOCl + H_2O \longrightarrow C_6H_5SO_2Cl + 2NaOH$$

still gives the same molecular proportions to the reaction as the first equation.

Chlorates present in the sodium hypochlorite solution used presented no problem, since they do not react with the sulfinate and may still be detected after completion of the oxidation.

TEST PAPER. The starch-iodide test paper used in the procedure described here must be very sensitive. The following formula, used in this laboratory, was found satisfactory:

One liter of distilled water is boiled several minutes, and 3 grams of pure cadmium iodide crystals are added to it, followed by 5 grams of potato starch which have been made into a thin paste with water. The mixture is boiled for several minutes. It is allowed to settle for at least 24 hours, and the clear liquor is decanted. Then strips of Reeves-Angel filter paper No. 211, about 12.5 cm. (5 inches) wide, are passed through the solution and dried in a relatively dust-free atmosphere, away from fumes. The strips are cut into thin strips 1.25×12.5 cm. (0.5×5 inches).

PROCEDURE

A sample, S, equivalent to approximately 2.5 grams of sodium benzene sulfinate is accurately weighed and transferred to a 250-ml. Erlenmeyer flask, diluted to approximately 150 ml. with distilled water, and made slightly alkaline with dilute sodium hydroxide solution, using a very small spot on brilliant yellow paper as an indicator.

Ten milliliters of 10% barium chloride solution are added and the precipitate of barium sulfite (and barium sulfate) is allowed to digest for 0.5 hour on a steam bath. The solution is then filtered, washed directly into a 250-ml. volumetric flask, and made to the mark at room temperature (Solution A).

Twenty milliliters of about 12% sodium hypochlorite solution are diluted to approximately 1 liter (Solution B). Fifty milliliters of this solution are titrated with standard 0.1 N sodium arsenite solution until the blue spot which first appears on starch–cadmium iodide test paper when touched by a drop of the solution ceases entirely to show. (This standardization of solution B must be repeated if the latter is to be used after several hours' standing.) Titration = b.

Fifty milliliters of solution B, 50 ml. of 10% sodium carbonate solution, and 100 ml. of distilled water are cooled to about 15° C. Into this solution, solution A is run from a buret until a blue spot ceases entirely to appear, as above. Titration = s.

CALCULATION

$$\frac{b \times 0.016416 \times 100}{2 \times \frac{s}{250} \times S} = \frac{205.2 \times b}{s \times S} = \% \text{ sodium benzene sulfinate}$$

EXPERIMENTAL

A good grade of sodium benzene sulfinate was recrystallized three times from water. The crystals obtained were dried over sulfuric acid in vacuum for 36 hours, then dried at 100° C. in air for 1 hour. The resultant compound was analyzed and found to contain S, 19.51%; C, 44.00%; H, 3.13%. Calculated: S, 19.52%; C, 43.89%; H, 3.07%.

This material was analyzed with the results shown in Table I.

To test further the relative values of the two procedures for commercial application, a mixture of the pure sodium benzene sulfinate with the inorganic salts usually found in the technical product, and sodium benzene sulfonate, a possible impurity, was

Table I. Analysis of Sodium Benzene Sulfinate

	Proposed Method	Ferric Salt Method
	%	%
1st run	100.24	99.66
2nd run	100.10	100.43
3rd run	100.10	95.94
4th run	99.97	....
5th run	99.97	....
6th run	100.11	....

Table II. Analysis of Mixture

	Proposed Method	Ferric Salt Method
	%	%
1st run	80.26	89.3
2nd run	79.89	98.3
3rd run	80.18	94.4
4th run	80.18	..
5th run	80.09	..
6th run	80.18	..

prepared. This combination was thoroughly ground and mixed to ensure homogeneity. Content of the mixture follows:

	Grams		Grams		Grams
NaCl	2.5000	Na_2SO_3	2.5000	$C_6H_5SO_2Na$	40.0000
Na_2SO_4	2.5000	$C_6H_5SO_3Na$	2.5000		

An analysis of the mixture containing 80.00% sodium benzene sulfinate gave the results shown in Table II.

ACKNOWLEDGMENT

The author is duly grateful to J. Werner for guidance, and to P. Nawiasky and R. H. Zappert for cooperation and constructive criticism.

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Determination of 1,2-Propylene Glycol in Ethylene Glycol

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A procedure for the determination of 1,2-propylene glycol in the presence of ethylene glycol consists of a periodate oxidation of 1,2-propylene glycol to acetaldehyde and formaldehyde and of ethylene glycol to formaldehyde, and the separation of the two aldehydes by a blowing-out process, forming the acetaldehyde sulfite addition compound which is titrated with iodine. By this method it is possible to determine 98% of the 1,2-propylene glycol present over a range of 0 to 90% 1,2-propylene glycol in ethylene glycol. A method for determining total glycols by periodate is also given.

A METHOD was desired for the determination of 1,2-propylene glycol in ethylene glycol, in varying amounts and in various types of samples. Fractionation procedures were impractical, since they required too much time and did not easily permit analytical separation because of the small difference in boiling points. Furthermore, this type of method would not be satisfactory when dilute aqueous solutions were encountered.

A search of the literature revealed much work on this problem, but none suitable to the authors' needs.

Hoepe and Treadwell (*4*) developed a method for the quantitative determination of glycerol, ethylene glycol, and 1,2-propylene glycol involving periodate oxidation of the alcohols and depending on accurate determination by the potassium cyanide method of formaldehyde in the presence of acetaldehyde. The authors found this procedure unsuitable, owing to interference of the acetaldehyde. In a method for determining small amounts of lactic acid in blood and urine, developed by Clausen (*1*), the lactic acid was oxidized to acetaldehyde which was removed by an aeration process from the other oxidized materials. Nicolet and Shinn (*6*) determined methyl pentose in the presence of pentoses by a periodate oxidation and separation of aldehydes by aeration. As the basis for the present work Shupe's (*7*) micro application of the Nicolet and Shinn procedure to glycols in cosmetic ingredients was used. Changes in design of equipment and procedure were made to ensure theoretical results in the type samples encountered.

OUTLINE

The oxidation of glycerol by the periodate method of Malaprade (*5*), Fleury and Fatôme (*3*) and adaptation to glycols by Denice (*2*) may be expressed as follows:

$$C_2H_4(OH)_2 + HIO_4 \longrightarrow 2CH_2O + HIO_3 + H_2O$$

$$CH_3CHOHCH_2OH + HIO_4 \longrightarrow CH_2O + CH_3CHO + HIO_3 + H_2O$$

The ethylene glycol yields two molecules of formaldehyde, while the 1,2-propylene glycol reacts to give one molecule each of formaldehyde and acetaldehyde. The aldehydes are separated by blowing them through a saturated solution of sodium bicarbonate containing a definite quantity of glycine. This treatment removes the formaldehyde, and the acetaldehyde is then determined by the sodium bisulfite procedure:

$$HCHO + NH_2CH_2COOH \longrightarrow CH_2NCH_2COOH + H_2O$$

$$CH_3CHO + NaHSO_3 \longrightarrow CH_3C(OH)(H)—SO_3Na$$

$$2NaHSO_3 + 2I_2 + 2H_2O \longrightarrow 4HI + H_2SO_4 + Na_2SO_4$$

The sulfite solution containing the absorbed acetaldehyde is treated with iodine to remove excess sulfite and then made alkaline with saturated sodium bicarbonate which destroys the addition compound. The sulfite thus liberated is titrated with a standard iodine solution with a buffer added just before the end point.

REAGENTS

Periodic Acid, 0.1 *M*. Weigh 10.7 grams of sodium metaperiodate into a 500-ml. volumetric flask, and add 200 ml. of water followed by 100 ml. of 1 *N* sulfuric acid. Dilute the solution to volume with distilled water and shake the flask until solution is complete.

Iodine, standard 0.1 *N*.

Iodine, standard 0.02 *N*. Dissolve 30 grams of potassium iodide in 100 ml. of water in a 500-ml. volumetric flask and measure in accurately from a buret 100 ml. of standard 0.1 *N* iodine. Make to volume with distilled water.

Iodine, approximately 0.1 *N*.

Sodium Bisulfite, 5%. Dissolve 5 grams of sodium bisulfite in 100 ml. of water.

Buffer (Borax–sodium carbonate mixture). Dissolve 4 grams of borax ($Na_2B_4O_7.10H_2O$) and 5 grams of anhydrous sodium carbonate in 100 ml. of water.

Glycine (aminoacetic acid). Dissolve 5 grams of U.S.P. glycine in saturated sodium bicarbonate solution and dilute to 250-ml. volume with saturated sodium bicarbonate solution.

Starch. Dissolve 0.5 gram of soluble starch in 10 ml. of cold water and add to 90 ml. of boiling water. Boil 5 minutes and cool.

Sodium Bicarbonate, U.S.P. powder.

Sodium Bicarbonate, saturated aqueous solution.

Sodium Arsenite, standard 0.1 *N*.

Potassium Iodide, approximately 10%.

APPARATUS

Special equipment used in determining 1,2-propylene glycol consisted of:

Carbon Dioxide or Nitrogen, one cylinder.

Flowmeter, calibrated for 1.5 liters of carbon dioxide per minute.

Reaction and Absorption Tubes. The equipment consists of a series of 4 test tubes connected in a manner satisfactory for passing gas through the solutions. The first tube (200 × 29 mm.) is fitted with a 3-hole rubber stopper carrying a small separatory funnel through which the sample aliquot and periodate solutions are added. This funnel also serves as an inlet tube for carbon dioxide. A large-bore glass tube, also inserted in the stopper of the first tube, is divided above the stopper by a piece of rubber tubing. It serves as a reservoir and inlet for the sodium

bicarbonate powder. A pinchclamp on the rubber tubing prevents loss of gas when the sodium bicarbonate is added. The other 3 tubes in the series are 150 × 25 mm.

PROCEDURE

Total Glycols. Pipet an aliquot of not more than 30 ml., containing not less than 15 mg. nor more than 90 mg. of glycols calculated as ethylene glycol, into a 125-ml. Erlenmeyer flask. Pipet in 15.00 ml. of 0.1 *M* periodic acid, mix well, and allow to stand 15 minutes. Add 30 ml. of saturated sodium bicarbonate solution, after which the solution should be approximately neutral. Measure in accurately 50.00 ml. of 0.1 *N* sodium arsenite and finally add 1 ml. of 10% potassium iodide and an excess of solid sodium bicarbonate. Titrate with 0.1 *N* iodine. With a little practice the yellow end point can readily be detected, and the use of this end point without the addition of starch is preferred. The presence of solid sodium bicarbonate improves the quality of the end point by ensuring a saturated solution.

Run a blank determination by placing 15.00 ml. of 0.1 *M* periodic acid in a 125-ml. Erlenmeyer flask, add 30 ml. of saturated sodium bicarbonate solution, 50.00 ml. of 0.1 *N* sodium arsenite, and 1 ml. of 10% potassium iodide, and allow the solution to stand 15 minutes. Add solid sodium bicarbonate in excess and titrate with 0.1 *N* iodine to the yellow end point.

The milliliters of 0.1 *N* iodine used for the determination minus the milliliter used for the blank gives the milliliter of 0.1 *N* iodine equivalent to the periodic acid used to oxidize the ethylene glycol and 1,2-propylene glycol.

$$\frac{\text{Net ml. of 0.1 } N \text{ iodine} \times 0.003102 \times 100}{\text{sample weight}} = \%\text{ by weight of ethylene glycol + 1,2-propylene glycol, as ethylene glycol}$$

The above result (total glycol as ethylene glycol), minus per cent of 1,2-propylene glycol from the following determination times 31/38, equals per cent by weight of ethylene glycol.

Glycerol, if present, must be determined and accounted for, since it is also oxidized by periodate in accordance with the following equation:

$$CH_2OHCHOHCH_2OH + 2KIO_4 \longrightarrow 2CH_2O + HCOOH + 2KIO_3 + H_2O$$

In this case the formic acid may be titrated.

1,2-Propylene Glycol. Accurately weigh a sample of the proper size and dilute to a suitable volume, so that a 10- to 20-ml. aliquot will contain not more than 50 mg. of total glycols and not more than 10 mg. as 1,2-propylene glycol. Pipet an aliquot to the largest test tube of the series described above and stopper it after adding water, if necessary, to make a final volume of 25 ml. Pipet into the second test tube sufficient glycine solution to leave a 10% excess of glycine over the amount required to remove the formaldehyde. Too large an excess will remove some acetaldehyde and cause low results. If the ethylene glycol content is unknown, make a trial determination first and calculate the amount of glycine from the result. Add to the second tube saturated sodium bicarbonate solution sufficient to make the final volume 10 ml. In the third and fourth tubes place 1 ml. of 5% sodium bisulfite and 15 ml. of distilled water.

Place 15 ml. of 0.1 *M* periodic acid in the separatory funnel and connect the carbon dioxide line. Open the stopcock and allow the acid to run into the reaction tube. Mix the solution gently for 15 minutes by passing in a small amount of carbon dioxide. Meanwhile place 4 grams of solid sodium bicarbonate in the large-bore glass tube. After the 15-minute mixing, remove the pinchclamp and tap in the sodium bicarbonate. Replace the clamp and pass in carbon dioxide at the rate of 1.5 liters per minute for 1 hour.

Transfer the contents of tubes 3 and 4 to a 250-ml. Erlenmeyer flask with the aid of a wash bottle. Add 5 ml. of starch indicator and run in the approximately 0.1 *N* iodine from a buret until a blue color persists, shaking the flask continually. Avoid the addition of a large excess at any one time. Discharge the blue color with a drop of 5% sodium bisulfite. After 5 minutes add 0.02 *N* iodine dropwise to the blue starch-iodine end point. Now add 10 ml. of saturated sodium bicarbonate solution and again titrate with 0.02 *N* iodine to the blue color. Before taking the final end point add 10 ml. of the borax-carbonate buffer solution. Record the total volume of 0.02 *N* iodine solution used after the excess sodium bisulfite was removed by the first addition of 0.02 *N* iodine, and calculate the per cent by weight of 1,2-propylene glycol as follows:

Table I. Analytical Data

Sample Weight	Ethylene Glycol Present	1,2-Propylene Glycol Present	1,2-Propylene Glycol Found by Acetaldehyde	Error
Mg.	%	%	%	%
43.98	95.44	4.56	4.59	+0.7
45.98	91.29	8.71	8.61	−1.2
47.98	87.50	12.50	12.50	±0.0
49.98	84.00	16.00	16.22	+1.4
25.85	61.10	38.90	38.16	−1.9
16.90	41.13	58.87	58.24	−1.1
12.61	20.65	79.35	78.22	−1.4
11.06	9.50	90.50	89.56	−2.1

$$\frac{\text{Ml. of 0.02}N \text{ iodine} \times 0.00076 \times \text{aliquot factor} \times 100}{\text{sample weight}} = \%\text{ by weight of 1,2-propylene glycol}$$

DISCUSSION

Various methods were tried to secure better results on the acetaldehyde determination. No noticeable difference was observed when the periodate oxidation was carried out at 0° and 100° C. (In order to keep the contents of the reaction tube at 100° C. without concentration, a condenser was used on the reaction tube.) Following the procedure of Nicolet and Shinn, alanine was used instead of glycine and 0.1 *N* arsenite solution was used to take care of excess periodate, without improving the method.

Best results were obtained by adding the periodate solution to the reaction tube through a separatory funnel and maintaining a closed system by adding the solid sodium bicarbonate through the large-bore glass tube.

That glycine, if present in too high a concentration, will remove some acetaldehyde was demonstrated by placing a glass ampoule containing a known amount of acetaldehyde in the reaction tube and going through the complete procedure. The recovery was 86%. Experimental results showed that formaldehyde was completely removed by the glycine; a 10% excess of glycine over that required to react with the formaldehyde formed proved to be satisfactory. In the lower percentage range of 1,2-propylene glycol very good recovery is obtained. However, in the higher ranges the results tended to run slightly low, since the glycine reacted with a very small amount of acetaldehyde.

The analytical data are given in Table I. The 1,2-propylene glycol used in this work was a regular Dow product and analyzed 100.8% by the periodate method. The ethylene glycol was Eastman Kodak Co.'s No. 133, analyzing 100.1% by the same procedure. Values given in Table I have not been corrected for the fact that the pure materials analyzed more than 100%.

Interfering substances are molecules containing adjacent hydroxyl groups or an amino group adjacent to a hydroxyl group.

An attempt was made to oxidize the formaldehyde to formic acid with hydrogen peroxide: the formic acid could then be determined by reduction of mercuric chloride to the insoluble mercurous chloride. Very high results were obtained, since hydrogen peroxide itself reduces mercuric chloride. However, when silver oxide was tried instead of hydrogen peroxide, the recovery was about 75%.

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Estimation of Salts of Weak Acids by Direct Titration in a Mixed Solvent

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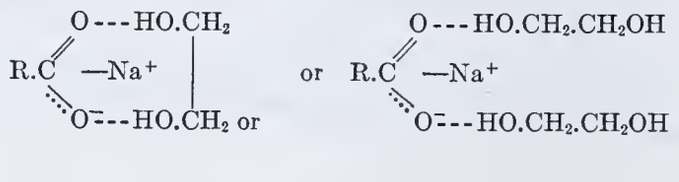
A simple volumetric method for the estimation of alkali metal salts of weak monobasic organic acids (—COONa), and a few weak monobasic inorganic acids, using direct titration in a mixture of cosolvents is described. Weak bases such as aniline, toluidine, and amines can also be titrated with sharp end points in the same media. The method consists of dissolving the salt in a mixture of solvents containing glycol and a higher alcohol or chloroform and titrating with hydrochloric or perchloric acid in the same mix-solvent, the end point being determined either potentiometrically or with the help of methyl red or methyl orange. In such media the order of indicator range is sometimes the reverse of that in an aqueous medium. Further applications of the method have been made or suggested for estimation of soaps, amino acids, weak acid–strong acid mixtures, and equivalent weight of any unknown organic acid.

THERE is no easy and accurate method for the estimation of salts of weak acids such as sodium acetate. Generally it is done (*7*, *13*) by distilling the acid liberated by sulfuric acid or phosphoric acid and absorbing it in standard alkali, or by the pyrolytic method. For salts of nonvolatile acids like sodium benzoate the matter is more complicated and may involve extraction from acid with ether. The present paper describes a simple direct method which is rapid and accurate, has some special features and advantages which are not met in aqueous solution, and is remarkably free from all complicating factors due to hydrolysis.

The method depends on the fact that all salts of monobasic weak organic acids containing the group COONa are highly soluble in a glycol or a glycol mixed with some solvent for hydrocarbons. Such mixtures may be referred to as G-H mixtures, where G stands for any glycol type of solvent and H represents any solvent for hydrocarbons, such as hydrocarbons themselves, alcohols, chlorinated hydrocarbons, etc. The solutions thus obtained can be titrated directly with hydrochloric or perchloric acid dissolved in the same solvent mixture, the end point being indicated either potentiometrically or by the color change of indicators. The method likewise can be used with a few weak inorganic acids, the salts of which are soluble in the aforementioned solvents, and can be applied to determine any excess base which might be present in a neutral salt, since no free base is produced by hydrolysis. The excess base which makes the solution alkaline to phenolphthalein or cresol red can be directly determined by titration in the G-H solvent medium. Thus, by a double indicator titration, first with phenolphthalein or cresol red and then with methyl orange or methyl red, both the free base and the combined base can be determined.

Weak acids are far less dissociated in nonaqueous solvents such as alcohol than in water; hence, their sodium salts act as bases towards indicators. However, most are only slightly soluble in such solvents and the end point is not sharp because the color change or the pH change extends over a long interval. The solvent medium here proposed provides high solubility and a sharp end point.

The COONa group in organic salts of the type R.COONa undergoes a specific solvation with glycols, evidently due to hydrogen bond formation as shown below, leading to a high solubility.



Of course, the —COO— group has no double bond in the classical sense as represented in the conventional formula, but both the oxygen atoms are equivalent, owing to a mesomeric shift in the electronic structure. If R is large enough it prevents adequate solvation of the whole molecule by the glycolic group alone, and only by the addition of a hydrocarbon solvent is a high solubility attained.

The solvent properties of G-H mixtures have been extensively studied and the theoretical aspects discussed in separate publications (*10*, *12*); here these mixtures will be discussed only in respect to their suitability as solvent media for titration. In conformity with the above idea it has been found that glycols or G-H mixtures are powerful solvents for any organic salt of the type R.COONa. This is illustrated by a few data in Table I, taken from a portion of a forthcoming publication on soap solubility in G-H solvents.

As can be observed, R can stand for both aliphatic and aromatic groups, and hence G-H mixtures might be regarded as an almost universal solvent for alkali metal salts of any monobasic organic acid. They serve as good solvent media for direct titration of the salts with strong acids.

Almost any G-H mixture can be used for these direct titrations, but in making the choice the author has paid attention to the following points: sharp end point, high solvent power, low viscosity, low volatility, freedom from toxicity, and easy availability. Of the three commercially available glycols, ethylene glycol is slightly preferable to propylene and to diethylene glycol in respect to the second and third points, but the advantage is not decisive. From the many solvents for hydrocarbons, isopropyl alcohol has been chosen as the cosolvent with ethylene and propylene glycol as best meeting all the above requirements. In extreme cases like sodium stearate, isopropyl alcohol does not confer sufficient hydrocarbon-dissolving property and hence other more powerful solvents, such as butyl or amyl alcohol, chloroform, dioxane, etc., might be used.

Table I. Solubility of Salts at 25° C. in Glycolic Media

(Grams of anhydrous salt per 100 grams of solvent)

Salt	Ethylene Glycol		Propylene Glycol	
	Alone	With cosolvent	Alone	With cosolvent
Sodium acetate	29.05	22.57[a]	.....	....
Sodium caprylate	29.52	....	22.31	....
Sodium laurate	11.92	....	12.19	....
Sodium oleate	16.43	28.81[b]	11.72	15.33[b]
Sodium benzoate	24.87	21.95[c]	13.13	10.24[a]
Sodium salicylate	Very high	....	Very high	....
Sodium propyl nitronate	39.51	34.89[c]	21.84	18.82[c]
Sodium nitrite	16.78	....	8.47	....
Borax	.....	....	....	9.05
Sodium metaborate	6.98	....	3.36	....

[a] Chloroform, 20%.
[b] Butyl alcohol, 50%.
[c] *n*-Amyl alcohol, 20%.

The proportion of isopropyl alcohol which can be satisfactorily used as a cosolvent for glycol extends over a wide range, from 15 to 70% by volume being satisfactory. The author has, however, used a mixture 1 to 1 by volume of ethylene or propylene glycol and isopropyl alcohol as a standard solvent medium; although 20 volume % of the latter is slightly better with respect to sensitivity and solubility, the low viscosity of the 1 to 1 composition is advantageous.

The two acids tried, hydrochloric and perchloric, give equally satisfactory results. The latter is preferred, particularly in

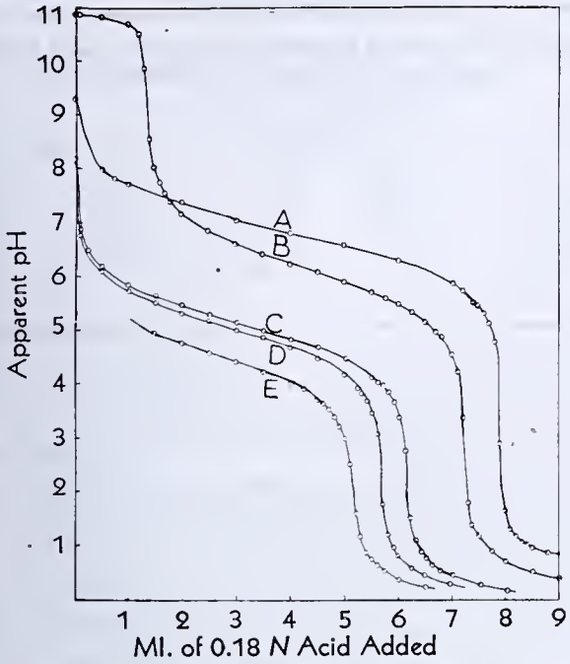


Figure 1. Potentiometric Titration Curves of Organic Acid Salts in G-H Solvents

0.18 *N* perchloric acid, glass electrodes
A. Sodium acetate
B. Sodium cinnamate containing excess alkali
C. Sodium formate
D. Sodium mandalate
E. Sodium salicylate

titrating concentrated solutions, since the perchlorate formed is much more soluble than the chloride and so does not produce any haziness during titration through separation of finely divided salt. This equality in strength, as evidenced by practically identical titration curves, is contrary to the findings of Hall and his co-workers (*5*), who found hydrochloric acid to give a steeper titration curve than perchloric acid in glacial acetic acid medium, and to the observations of others (*2*, *3*, *6*) on acid strength in nonaqueous solvents. That the intrinsic strength of perchloric acid should be more than that of hydrochloric acid is easily understood, since the coordinate bonds of the chlorine ion to the oxygens are formally equivalent to a transfer of negative charge resulting in a higher protogenic tendency (basic power) of the resulting perchlorate ion. However, this leveling effect on acids of glycolic solvents, which have very weak acidic or protogenic power but possibly no basic or protophilic power, is difficult to understand unless we admit that not only the acidic or basic nature of the medium but also its solvation power is an important factor. This may cause in some cases, as in the case of indicators, a reversal of the normal order of acid strengths.

Potentiometric titrations were conducted satisfactorily using a Beckman glass electrode. The readings on the dial of the pH meter indicate, of course, only apparent pH values, because the scale of acidity has not been thermodynamically established for these solvents, and because of the unknown value for the potential at the liquid junction between the solvent and the saturated potassium chloride solution surrounding the reference electrode. Nevertheless, it is remarkable that the pH changes and the behavior of the indicators are nearly in the same range as in an aqueous medium in weakly acid region. In strongly acid region the apparent pH is much lower; in fact, it is negative in a decinormal acid solution and hence, except as a means of determining the end point, these values have no significance at all.

A few titration curves are shown in Figures 1 and 2. The change at the equivalence point is rapid, and therefore the end point can be ascertained with great accuracy. For comparison, a titration curve of sodium acetate in water is shown in Figure 2. It will be observed that pure glycol could be used as a solvent medium with slight sacrifice of accuracy so far as the steepness of the titration curve is concerned. But two other additional factors stand in its way to be used as such: (1) its viscosity is rather high, which introduces a large "drainage" error in delivering from a buret, and (2) many organic acid salts are insoluble or sparingly soluble in the pure glycol but are sufficiently soluble in suitable G-H mixtures.

The same titration can be done with the help of suitable acid-base indicators. Owing to the uncertainties in the significance of conventional pH measurement in organic solvents, the choice of indicator has to be made by trial and error. Both methyl red and methyl orange can be satisfactorily used. The latter gives a sharper end point, but the former gives a brighter one. In more than 300 titrations by this method in connection with measurements of soap solubility, using both the indicators, methyl red was found more convenient, owing to a greater sensitivity of the eye in judging the methyl red color. The colors of the indicators are somewhat different from those in water and are much brighter and more contrasted. The color on the acid side is bright pink and that on the alkaline side is canary yellow for both indicators.

Of all common solvents, only a few as listed below show bright color and sharp change from alkaline to strong acid with methyl red, whereas in all other solvents a dull brownish orange color with a sluggish change is observed. The suitability of the solvent was judged by a very simple test: Five milliliters of each solvent were taken in each of two separate test tubes and to one was added a drop of glacial acetic acid and to the other a drop of alcoholic 0.2 *N* hydrochloric acid. Obviously, the solvents which show color with acetic acid and/or fail to show sharp change with hydrochloric acid cannot be used. The following solvents more or less qualified in the above test: propylene glycol, diethylene glycol, ethylene glycol, benzyl alcohol, methyl alcohol, phenols, chloroform, methylene chloride, glacial acetic acid, and cyclohexanol. The first two solvents are found best for the purpose, followed very closely by the third. The solvents at the end of the list are rather poor for the purpose. With somewhat less accuracy benzyl alcohol and methyl alcohol probably can be used in place of glycols, if other conditions permit. In fact, with respect to the brightness of the acid color of methyl red, benzyl alcohol (and phenol) is definitely superior to even the glycols, but its low solvent power, less availability, presence of blanks against alkali in even the purest commercial variety, etc., forfeited its chance of being considered for this purpose. If phenols, cresols, benzyl alcohol, etc., become available commercially in purer state they would perhaps be usable in place of the glycols

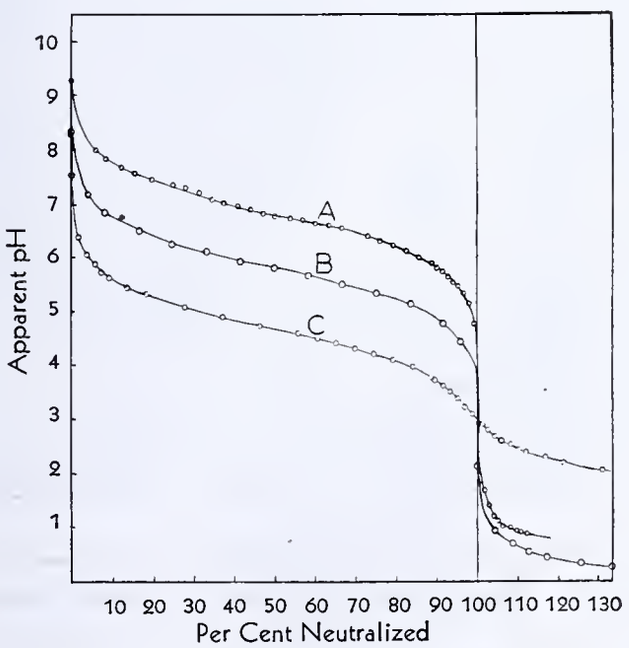


Figure 2. Potentiometric Titration Curve for Sodium Acetate

0.18 *N* perchloric acid, glass electrode
A. G-H solvent *B.* Glycol *C.* Water

for such titrations. As cosolvents ethyl alcohol shows too sluggish a color change to be used, whereas higher alcohols are better.

EXPERIMENTAL

Estimation of Salts of Organic Acids. Potentiometric Method. Since the use of the glass electrode under such conditions is a departure from the usual procedure, a rather detailed description of the procedure is given.

All the potentiometric titration curves were obtained by dissolving 0.1 to 0.2 gram of the salt (equivalent to 8 to 10 ml. of 0.18 *N* acid) in 20 ml. of the G-H solvent and titrating with 6 *M* perchloric acid, using a Beckman glass electrode (Type 1190) with a Beckman pH meter. The solution to be titrated was taken in a 50-cc. beaker which was stirred by an electromagnetic device with a small piece of thin glass tubing containing a sealed iron nail. The glass electrode was adjusted with a buffer of pH 7, washed with a spray of distilled water, which was carefully tissued off completely with very soft absorbent paper, and immediately placed in the solution. The type of calomel electrode whose connection was made through a sealed-in thin asbestos fiber was used and was treated in the same way as the glass electrode. In less than 2 minutes very steady readings were obtained and the titration was then carried out as usual with perchloric acid dissolved in the same solvent mixture delivered from a 10-cc. buret. The pH value was checked with the same buffer after titration. It was found that the buffer now gave a value always higher by 0.02 to 0.06 pH unit, which tended to disappear on keeping the electrode immersed for a long time.

In order to determine how far these apparent pH values are reproducible, a solution of sodium acetate–acetic acid (approximately decinormal with respect to both components) in standard G-H mixture was made and its apparent pH was determined from time to time (Table II). The values are fairly reproducible and comparable.

Sodium salts of all the following acids have been found to give satisfactory results: acetic, propionic, butyric, oleic, stearic, benzoic, cinnamic, mandelic, and lactic. It would appear from the titration curve of formic acid that this is about the limit of acid strength suitable to the method with high accuracy, and salts of acids much stronger than formic ($K_a = 2.1 \times 10^{-4}$) cannot be titrated without sacrificing accuracy.

Indicator Method. From 0.2 to 1.0 gram of the salt is dissolved in about 10 to 15 cc. of the solvent mixture in a 50-cc. conical flask or a big test tube (1 × 5 inch) and is titrated with 0.18 *N* perchloric acid, or stronger if necessary, to keep the titer to a convenient volume. In order to effect a quick dissolution of the solid it is advisable first to add 5 cc. of propylene glycol to moisten it fully, allow a few minutes for swelling, warming if necessary, and then add about an equal volume of the other solvent (usually isopropyl alcohol or chloroform) and stir to get a clear solution. Three to 5 drops of 0.05% alcoholic indicator solution are added and the solution is titrated slowly with standard perchloric acid solution in propylene glycol–isopropyl alcohol mixture (1 to 1 by volume) until the color changes sharply to a bright pink. To facilitate the detection of color change the conical flask is placed on a white background and the acid is added near the end point in steps of 0.02 cc. No blanks are necessary even with the commercial solvents (excepting chloroform), which are neutral and give a bright pink color on adding a drop of the acid. The chloroform, if it contains free hydrochloric acid, should be washed with water and dried before use. The presence of benzyl alcohol, phenol, or any benzoid compound, if added, brightens the color of methyl red.

Accuracy of the Method. It is necessary to check the method using a pure salt of an organic acid. Unfortunately, such chemicals are not easily available or prepared because these salts have a tendency to crystallize from water with excess acid formed by hydrolysis. In order to avoid any such doubt the following procedure was used to check the proposed method.

Table II. Apparent pH of an Acetate Buffer in G-H Solvent

Type of Electrode	Days	Apparent pH
Type 1190	0	6.33
	1	6.31
	2	6.32
	3	6.30
Type 1190 (a different glass electrode)	3	6.31

Table III. Titration of Sodium Hydroxide in G-H Medium Containing an Excess of an Organic Acid

Organic Acid	Volume of 0.2 *N* Acid Required: Actual	Observed	Error: *Ml.*	%
	Ml.	*Ml.*		
Acetic	8.45	8.43	−0.02	−0.24
Propionic	8.41	8.41	0.00	0.00
Butyric	8.43	8.39	−0.04	−0.48
Benzoic	8.45	8.43	−0.02	−0.23
Cinnamic	8.45	8.46	+0.01	+0.12
			Mean	−0.17

A solution of sodium hydroxide (approximately 0.14 *N*) was made in ethylene glycol. An equal volume (about 12 cc.) of this solution and isopropyl alcohol was mixed. The isopropyl alcohol contained an excess (about 10%) of an organic acid and hence the resulting solution is the same as a solution of a salt of an organic acid containing a known amount of the same in the presence of an excess of the same acid. This solution was potentiometrically titrated as described above. To avoid any drainage error due to high viscosity of glycol, the amount of glycolic caustic soda used was always determined by weighing. The results obtained are shown in Table III, from which it will be observed that the method is capable of an accuracy of at least 1 part in 500 without any special technique.

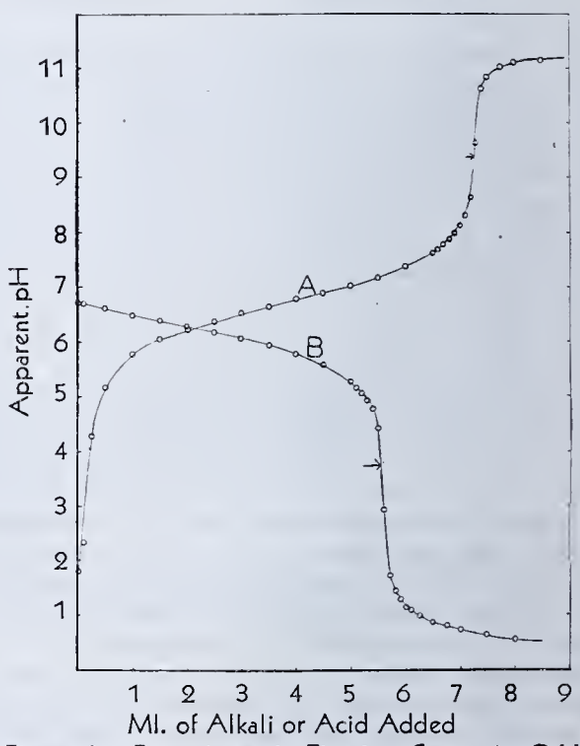


Figure 3. Potentiometric Titration Curves in G-H Solvents, Using Glass Electrode

A. Boric acid by 0.2 *N* alkali
B. Borax by 0.18 *N* perchloric acid

Estimation of Salts of Inorganic Acids. It has been found that not only the COONa group but any sodium salt containing the group *X*OONa, where *X* stands for any negative element, is highly soluble in G-H mixtures and can be titrated accurately in such media. The inorganic salts containing this requisite structure are metaborates, aluminates, nitrites, nitronates, hypophosphites, hyposulfites, sulfinates, chlorites, etc., all of which are highly soluble in glycols and G-H mixtures, and they can be titrated as described above by either the potentiometric or indicator method. The only difficulty is encountered with strongly reducing hyposulfite which tends to decolorize the indicator, but in the 1 to 1 G-H solvent the reducing action is not sufficiently rapid to interfere with the titration. In fact, it has been observed that the G-H solvents, containing higher alcohols,

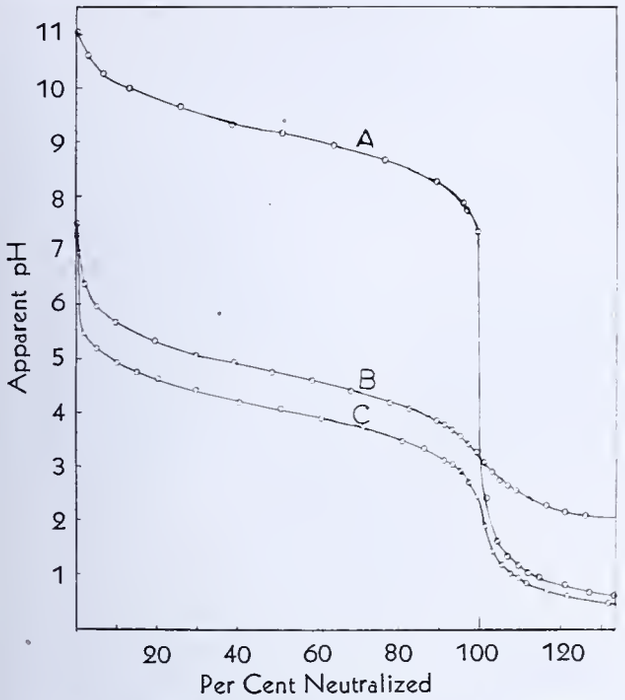


Figure 4. Potentiometric Titration Curves of Weak Bases in G-H Solvents

0.18 *N* perchloric acid

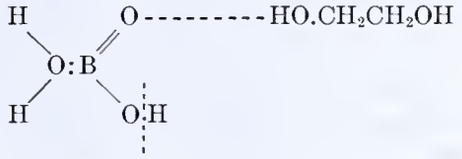
A. Ammonia *B.* Aniline in water *C.* Aniline

chloroform, etc., act as a retardant for such reduction processes, whereas in pure glycol the reduction is rapid.

In addition to the above acids there are a fair number of monobasic weak inorganic acids whose salts are sufficiently soluble in G-H mixtures to admit of such a titration. Such compounds are borate, silicate, arsenate, etc., which can be estimated in the same manner.

Estimation of Borates. In glycolic solvents, boric acid behaves as a moderately strong monobasic acid just as it does in aqueous solution in the presence of a large concentration of mannitol or glycerol. Hence, all the alkali-metal borates which have been found to be very highly soluble in glycolic solvents can be easily estimated. The salt is dissolved in glycolic solvent and is titrated with glycolic alkali to the color change of phenolphthalein or cresol red which gives the free acid equivalent. This solution is now titrated with glycolic perchloric acid using methyl red as indicator, which will give the total boric acid equivalent, provided no other weak acid is present in the system. The alkali titration of boric acid or borates gives a very sharp end point, whereas the acid titration is somewhat less accurate, owing to the fairly strong acidity of boric acid in glycolic solvent, making the end point less sharp. This is seen from the potentiometric titration curves of boric acid against alkali and borax against acid (Figure 3). The strong buffering effect of solutions of boric acid in the presence of borates is also worthy of notice.

The explanation of this behavior of boric acid can be given in terms of Lewis' theory. The octet structure of the boron in boric acid is not complete and the former can therefore accommodate two more electrons. Hence it is a monobasic acid (electron acceptor) like boron trichloride. Water forms a coordinate bond by lending a lone pair from its oxygen shell, which is equivalent to giving a formal negative charge to the central atom which inductively holds all the positive hydrogen atoms tighter. Alternatively, we might consider the structure of boric acid as shown below, $HBO_2.H_2O$, where it has a filled octet instead of a sextet as in the other structure, $B(OH)_3$. Glycol solvates the electronegative oxygen atom through hydrogen bond formation, as a result of which the hydroxylic hydrogen of boric acid becomes more prone to dissociation, similar to the stepwise behavior of a dibasic acid. This explanation, however, is different from that of Boeseken and co-workers (*1*), who consider that a coordinate compound is formed with a central boron atom after elimination of water between the hydroxyl groups of boric acid and glycol.



Estimation of Weak Bases. Since glycol is an acidic or protogenic solvent we should expect it, according to the Lowry-Brønsted theory, to augment the apparent basic power of a base, and beyond a certain strength make all bases appear strong. However, the acidic power of glycol will be inferior to water as a titration medium for weak bases. There is, however, one point to consider which makes it decidedly superior to water for such use. Water, owing to its amiphiprotic nature, also shows its protophilic activity on the salt formed during titration and hence obscures the end point. Thus, in titrating aniline in aqueous medium, we have two reactions simultaneously occurring, as shown below:

$$C_6H_5NH_2 + H^+ \longrightarrow C_6H_5NH_3^+$$

$$C_6H_5NH_3 + H_2O \rightleftharpoons C_6H_5NH_2 + H_3O^+$$

whereas in glycolic medium the second reaction, in which the solvent competes for the proton, will be much reduced.

A few potentiometric titration curves for weak bases are shown in Figure 4. It will be observed that aniline ($K_\beta = 4.6 \times 10^{-10}$ in water) gives a curve which is of almost the same type as that of ammonia in water; in other words, its apparent dissociation constant as judged by the steepness at the inflection point has been increased more than 10,000 times that in water. For comparison, its titration curve in water under the same conditions has been shown. It should be noted that the apparent pH of aniline in the G-H solvent titration curve is much lower than the same curve in water, whereas in the case of sodium acetate the apparent pH is higher before neutralization and lower after neutralization (Figure 2). *n*-Butylamine (mono) and ammonia have been found to give practically strong base curves in glycolic solvent.

Table IV. Estimation of Weak Bases by Direct Titration

Base	Taken *Gram*	Found *Gram*	Error %	Sharpness of End Points
Aniline	0.2594	0.2587	Mean error	Fairly sharp
	0.2034	0.2034	−0.16	
	0.1785	0.1776		
	0.2150	0.2154		
Strychnine	0.6525	0.6531	.....	Very sharp
Brucine	0.7821	0.8762[a]	.....	Extremely sharp
		0.7408[b]		
Pyridine	0.2392	0.2095	−12.42	
	0.1993	0.1705	−14.09	Poor
p-Toluidine	0.3040	0.3124	+ 2.7	Fair
Naphthylamine	0.3181	0.3674	+15.8	Poor
Quinoline	0.4309	0.4045	− 6.5	Fair

[a] Calculated as $4H_2O$.
[b] Calculated as anhydrous.

The end point can also be detected with the help of indicators as in the foregoing cases, methyl red being satisfactory for the purpose. The following weak bases were found to give fairly sharp end points with methyl red, the stronger base showing a sharper color change: aniline, quinoline, pyridine, β-naphthylamine, *p*-toluidine, and phenylhydrazine. Phenylhydrazine and to a less extent β-naphthylamine were found to destroy the indicator, probably by a slow chemical reaction, and could be titrated only by conducting the titration very quickly. The actual values could not be checked against titration values, since none of the above bases was obtainable in the analytically pure state, but with fairly pure samples of aniline (Merck, A.C.S. specification) the results agreed within 1% as shown in Table IV. Some of

the bases like toluidine and naphthylamine show high results, perhaps due to the presence of diamines as impurities.

The experimental procedure followed in these cases was to dissolve about 0.2 to 0.5 gram of the base in a mixture of 5 cc. of chloroform and 5 cc. of propylene glycol and to titrate the solution with the standard hydrochloric acid solution as in the previous cases, adding the solution in steps of 0.05 cc. near the equivalent point. Chloroform was used as cosolvent, as it was found that its presence gave a slightly better color change. The dissociation constant of the base in aqueous solution is not a reliable guide as to its suitability in the direct titration, because aniline, though a weaker base than pyridine, was found to give much sharper color changes and a steeper titration curve than the latter. In fact, it is impossible to judge the end point accurately with pyridine by this procedure unless by comparative methods. The pyridine used was an ordinary dry sample which gave about 10 to 12% lower results, which is perhaps due to the presence of its homologs.

Alkaloids like strychnine and brucine gave extremely sharp end points and in the former case, where a pure sample (Merck) was available, the result agreed very well.

Estimation of Salts of Dibasic Acids. Generally speaking, dibasic acids are not amenable to this direct titration for two reasons: (1) their salts are not sufficiently soluble in G-H solvents, and (2) for salts like oxalates and phthalates the free acid liberated during titration is strong enough to effect a color change of the indicators. It might be possible to titrate some of the weaker ones by this method. Strangely enough, hydrazine hydrate, which is dibasic, also could not be titrated in glycolic solvent with accuracy and the potentiometric titration curve shows an almost constant slope over a wide range near the equivalence point.

Choice of Indicators. The suitability of an indicator cannot be judged from these potentiometric curves in nonaqueous solvents and the best indicator for any titration is found only by empirical means. For example, butylamine, which registers an apparent pH of 11.3 in glycolic solvents, shows only a faint alkaline color with phenolphthalein, whereas in water a bright pink color is shown though the pH is only slightly higher (11.45). In fact, phenolphthalein in glycolic solvents is much less bright than in water and methyl red is just the reverse. The author believes that solvation plays a very important role and the color of dyes and indicator is largely influenced by the solvating power of the medium. It has been shown that glycol has a strong solvating power through hydrogen bond formation for $RCOO^-$ ion but has very little power for dibasic acid salts. This might be responsible for the selectivity of the indicators as noted above. Cresol red has been found to have a brighter color than phenolphthalein with the same color change interval and so can be used with advantage in place of phenolphthalein for free alkali titrations.

Even the order of indicator range might change with the nature of the solvent, though usually acids of the same type are nearly equally affected by change of solvent (*4*, *9*). A very striking case observed in G-H medium is the relative range of bromocresol green (pH 3.8 to 5.4) and methyl red (pH 4.4 to 6.2); the figures in parentheses are the color change interval in water. If we prepare a set of buffer solutions in G-H mixtures by partially neutralizing aniline with gradually increasing quantities of perchloric acid it is observed that bromocresol green changes to acid color with about one-third neutralization of aniline, while methyl red requires free acid to be present; though according to the values quoted above for an aqueous medium the reverse is to be expected. Bromophenol blue (pH 3.0 to 4.6) has also been observed to change to acid color at a higher level of pH than methyl red, but to a smaller extent than bromocresol green. That the titration range of sulfonphthalein indicators tends to shift to higher pH values in glycolic solvents is found in a recent work of Masi and Knight (*8*), who found that in 20 to 80 by weight glycol-water mixture the pK of bromothymol blue (dibromothymol sulfonphthalein) is 7.42 and in 40 to 60 glycol-water mixture it becomes 7.69 as against 7.21 in pure water.

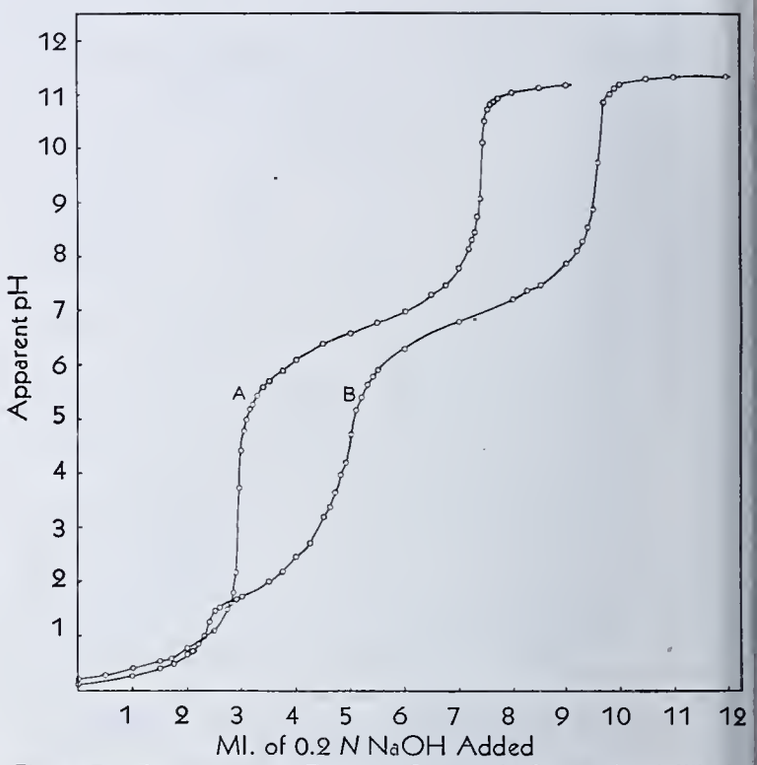


Figure 5. Potentiometric Titration Curves of a Strong Acid–Weak Acid Mixture in G-H Solvents

0.2 *N* sodium hydroxide
A. Nitric acid–acetic acid mixture
B. Sulfuric acid–acetic acid mixture

APPLICATIONS

Some interesting applications of this method can be made in diverse fields. Soap, as well as the free alkali present in it, can be conveniently measured. A very interesting application is in the estimation of amino acids, where the results are much more complicated because of the dipolar nature of their ions. The results of this method with reference to soaps (*11*) and amino acids and proteins will form the subject of separate publications.

Another suggested application is the estimation of a mixture of a weak acid and a strong acid in a single titration by a double indicator method or a potentiometric method. The possibility of such a method is shown by a typical titration curve of a mixture containing nitric acid and acetic acid by sodium hydroxide in G-H medium (Figure 5, *A*). Two sharp inflections at about apparent pH 4 and 10 are obtained corresponding to the neutralization of the nitric acid and the acetic acid, respectively. Such estimation is frequently necessary in industry with acid mixtures containing sulfuric acid, for example in the manufacture of cellulose acetate. Unfortunately, sulfuric acid is not very well amenable to this method because it does not behave as a very strong acid like the other mineral acids in G-H medium. This is shown by a titration curve of a mixture containing sulfuric acid and acetic acid by sodium hydroxide in G-H medium (Figure 5, *B*). Surprisingly, sulfuric acid, unlike in water, shows two inflections corresponding to its two dissociable hydrogen atoms. The first neutralization occurs around apparent pH 1.5 and is not very sharp, whereas the second or complete neutralization occurs around apparent pH 4 and the latter inflection is only moderately sharp, far less than the other mineral acids. The third inflection occurs as usual around apparent pH 10, corresponding to the complete neutralization of all acids. A better method in such case will be to determine the total acid by neutralization with sodium hydroxide in water or alcohol and then to estimate the sodium salt of the organic acid by direct titration in G-H medium after evaporating off the water or alcohol used for the above total acid titration.

Another application could be made in the determination of the equivalent weight of an organic acid without isolating it in the pure state. The sodium salt of the acid could be treated in aqueous or aqueous-alcoholic medium with excess of calcium chloride solution and the precipitated calcium salt dried and its equivalent weight determined by direct titration in G-H medium. A slight change of procedure is convenient here. The metallic soap is to be treated with chloroform first and after a little swelling is treated with propylene glycol to effect solution. The solution can then be titrated as usual. A sample of lauric acid (Eastman Kodak) treated by this method gave as equivalent weight for the calcium salt, 220.5 (theoretical, 219.23).

Various other applications, depending on the basic fact that we can clearly distinguish analytically a strong acid from a weak acid and a free base from a combined base by direct titration in a glycolic medium, can be easily made in other suitable cases.

CONCLUSIONS

The subject of acidimetry and alkalinity in organic solvents has hitherto not been systematically pursued. Data indicate that highly interesting and useful results are obtainable from this field and a long way is to be gone before these results can be brought in line with the standard data obtained in water, so that a unified theoretical presentation is possible. Two types of study are needed. First, it is necessary to fix up a pH scale in glycolic solvents with the help of a reversible hydrogen electrode and, second, we must have data about the performance of the usual acid-base indicators in the common organic solvents with respect to their sensitivity to all types of acids and bases. The glycolic solvents and their mixtures particularly recommend themselves for our first study, since they behave more closely to water than any other organic solvent, have fairly high solubility for salts, and are expected to be well suited to the functioning of reversible electrodes. The prospect seems definite that such a study will produce many newer and better methods of analysis and a more thorough understanding of the behavior of solvents.

Another fruitful line of study involving organic solvents in analytical chemistry remains practically untouched. The precipitation in gravimetric analysis is often attended with colloid formation and adsorption, to obviate which careful control of pH and other conditions is necessary. It is likely that by addition of suitable organic solvents better and easier control might be achieved, as in some cases of electrode position. It is also imaginable that some easier methods of separation and analysis using some organic solvents as the medium might come out of such studies.

ACKNOWLEDGMENT

The author gratefully acknowledges his indebtedness to James W. McBain for his valuable help, advice, and suggestions throughout the course of the work. Thanks are also due to George G. Manov for helpful criticism of the original manuscript, and to J. M. Luck for lending some of his apparatus.

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Determining the Moisture Equilibrium Curves of Hygroscopic Materials

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THE wartime packaging of rations and foods for the armed services and civilians has required extensive use of paper, paperboard, and other flexible packaging materials. The protection of moisture-sensitive products by nonhermetically sealed containers has aroused increased interest in the relationship of moisture content to equilibrium relative humidity. In anticipation of such work at the institute, a new procedure for obtaining sorption data was developed, the general principle of which was described by Gane (*2*). The method requires a minimum of uncommon apparatus, conditioning equipment, and technique. Specimens of adequate size may be used and data for complete sorption and desorption isotherms may be obtained simultaneously.

The procedure consists in suspending specimens in small closed vessels in which the relative humidity is controlled by saturated salt solutions. The number of test units is determined by the number of points desired on the sorption isotherms and an appropriate number of salt solutions is used. The specimen is suspended in the vessel by a rod which passes through a hole in the cover of the vessel and can be attached to the pan hook of an analytical balance to permit weighing the specimen without removal from the conditioned atmosphere. The hole in the top of the dish is sealed except during the weighing operation. All the operations are carried on in a room of controlled temperature.

Figure 1 is a photograph of a glass test unit in weighing position in a balance. The left-hand balance pan has been removed and a can with an appropriate slot has been inverted over the pan arrest to act as a platform for the unit.

Figure 2 is a schematic diagram of the glass test unit in normal storage position.

The specimen is normally held in a 93 × 18 mm. Petri dish and is uniformly distributed over the bottom of the dish. This dish is supported by a bracket made from 0.6-cm. (0.025-inch) aluminum sheet. The bracket is 8.1 cm. (3.25 inches) long and 1.25 cm. (0.5 inch) wide; it is bent to fit the dish snugly. This bracket is fastened to the threaded end of a 0.23-cm. (0.092-inch) diameter brass rod by means of two 2–56 brass nuts. The top

Table I. Equilibrium Relative Humidities for Saturated Salt Solutions

Chemical	Formula	Relative Humidity, % 73° F.	86° F.	100° F.
Ammonium phosphate	$NH_4H_2PO_4$	92.9	92.0	91.1
Potassium chromate	K_2CrO_4	86.5	86.3	85.6
Ammonium sulfate	$(NH_4)_2SO_4$	80.1	79.6	79.1
Sodium chloride	NaCl	75.5	75.2	75.1
Sodium acetate	$NaC_2H_3O_2$	74.8	71.4	67.7
Sodium nitrite	$NaNO_2$	64.8	63.3	61.8
Sodium bromide	NaBr	58.5	56.3	53.7
Sodium dichromate	$Na_2Cr_2O_7$	54.1	52.0	50.0
Magnesium nitrate	$Mg(NO_3)_2$	53.5	51.4	49.0
Potassium nitrite	KNO_2	48.6	47.2	45.9
Calcium nitrate	$Ca(NO_3)_2$	51.8	46.6	38.9
Potassium thiocyanate	KCNS	46.6	43.7	41.1
Potassium carbonate	K_2CO_3	43.9	43.5	43.4
Chromium trioxide	CrO_3	39.2	40.0	40.2
Magnesium chloride	$MgCl_2$	32.9	32.4	31.9
Potassium acetate	$KC_2H_3O_2$	22.9	22.0	20.4
Lithium chloride	LiCl	11.1	11.2	11.1

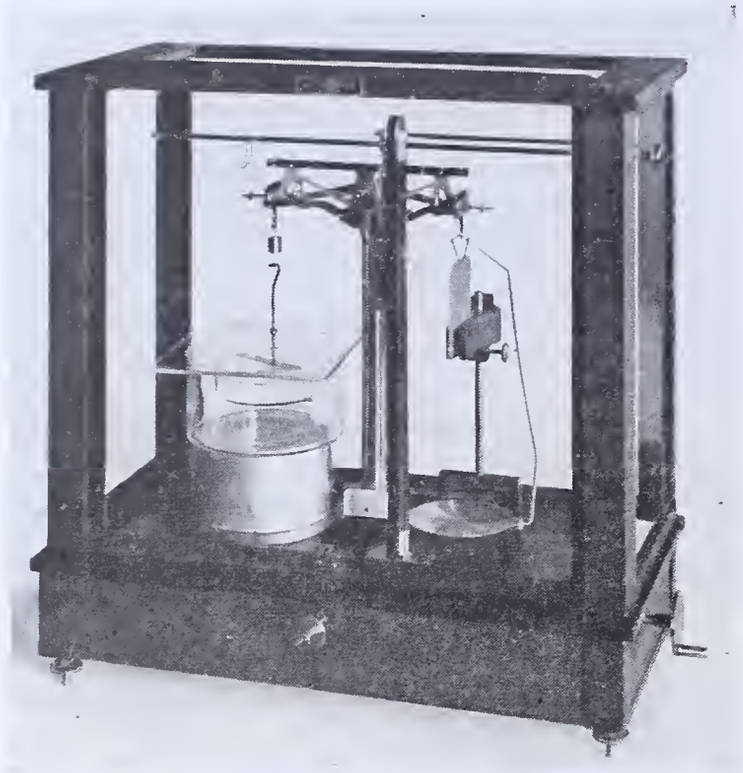

Figure 1. Glass Test Unit in Weighing Position

end of the rod is bent into a flat hook for convenient suspension from the pan hook of a balance or from the counterpoise used with a magnetic damping device, as shown in Figure 1. The over-all length of the rod, 8.1 cm. (3.25 inches), is arbitrary and depends upon the depth of the test unit and the height of the platform upon which the unit rests when in the balance. A brass cone is drilled, slipped on the rod, and soldered in place with the small end down and at a distance of 2.5 cm. (1 inch) from the bottom end of the rod. This cone is cut from a 0.6-cm. (0.25-inch) brass rod and has a half-angle of approximately 30°.

The top of the test unit is a 13.75-cm. (5.5-inch) square of double-strength window glass. A 0.47-cm. ($^3/_{16}$-inch) hole is cut 0.6 cm. (0.25 inch) off center on a line perpendicular to one edge of the plate and passing through its center. This off-center position is necessary because of space limitations in a typical analytical balance. In particular cases, it may be necessary to position this hole farther from the center of the plate. The brass cone is normally seated in the hole of the glass plate to seal the hole against passage of water vapor and to support the suspended system. The top edge of the hole on which the cone rests should be free from serious flaws. If a chipped edge results from the drilling operation, the edge should be ground lightly with Carborundum, using a brass cone of the type described. No sealing grease is employed in the zone of contact between the brass cone and the hole in the glass cover. The slight leak of water vapor through the zone has a negligible effect on the relative humidity in the vessel.

The main body of the test unit is a 125 × 65 mm. crystallizing dish. The top edge of the dish is ground on a flat surface—e.g., plate glass—with Carborundum to remove irregularities. The ground edge is lightly greased with Celvacene (Distillation Products, Inc.). This film of grease effects a good seal between the ground edge of the crystallizing dish and the glass plate when the unit is subsequently assembled.

Approximately 40 cc. of a saturated salt solution, having the desired equilibrium relative humidity, are poured into the bottom of the crystallizing dish. An excess of the salt is transferred to the dish to ensure a saturated condition of the solution while the test is carried out. In obtaining data for a desorption isotherm, where moisture is removed from the specimen and transferred to the saturated solution, it is advisable to have mounds of the excess salt exposed above the level of the solution.

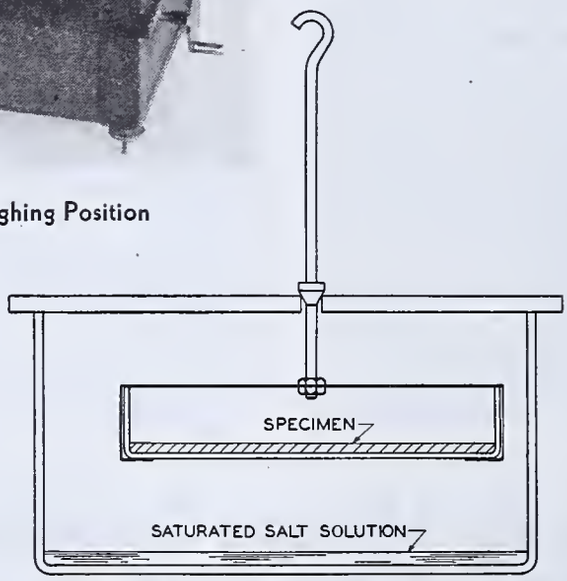

Figure 2. Schematic Diagram of Glass Test Unit

The glass test unit permits visual inspection of the specimen during the test; this is a convenience in testing foods and other materials for which the caking point, or other critical behavior, is of interest.

Table I lists the equilibrium relative humidities for a number of saturated salt solutions at 73°, 86°, and 100° F. (*1*).

PROCEDURE

The number of specimens and their initial conditioning vary with the purpose of the test. If a complete sorption isotherm is contemplated, it is necessary to dry the specimens, before assembling the test units, to a moisture content lower than that corresponding to the first point desired on the curve. The number of specimens depends on the number of points considered desirable in defining the isotherm. For many purposes, six to ten points are sufficient. A corresponding number of saturated salt solutions having equilibrium relative humidities appropriately distributed through the range from 10 or 15% to somewhat over 90% are needed. If a desorption isotherm is wanted, the specimens must be wet or conditioned at a high relative humidity before beginning the tests. On occasion, complete isotherms are not needed; the purpose is served by determining the moisture contents at two or three different relative humidities.

Since the detailed procedure for obtaining sorption data depends largely upon the purpose of the test and the type of material to be tested, it is beyond the scope of this discussion to cover the many phases involved in obtaining such data. The following discussion is therefore limited and presents only the procedure for the assembly and weighing operation of the test units.

A unit is temporarily assembled to obtain its tare. An empty Petri dish is placed in the bracket of the suspending device and the glass-plate cover is placed on a crystallizing dish. The zero of the balance is noted and adjusted if necessary. The left-hand pan is removed from the balance and the platform is placed in position straddling the pan arrest. The unit is placed on the platform and the hooked rod is lifted and gently engaged with the pan hook or with the eye of the counterpoise weight. The unit is then adjusted in position on the platform until the rod hangs freely through the hole in the glass plate. The combined weight of the suspending device and the empty Petri dish is then determined and recorded. The rod is unhooked and the unit is removed from the balance.

To insert the specimen and start the test, the cover of the unit is removed, the Petri dish is taken from the bracket, and a specimen is evenly distributed over the bottom of the dish. For materials which are very hygroscopic it may be desirable to replace the Petri dish with a shallow weighing bottle. Then the moisture content of the specimen can be determined in the same dish which is used to obtain the sorption data. The Petri dish is returned to its bracket, a film of grease is applied to the ground edge of the crystallizing dish, and the saturated salt solution, giving the desired relative humidity, is poured in the bottom of the crystallizing dish. The cover is replaced on the crystallizing dish and pressed lightly into continuous contact with the greased ground edge. The unit is then placed on a storage shelf until time for weighing. The specimens in the test units are weighed in the manner described at approximately 24-hour intervals until the weights become constant.

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Simultaneous Determination of Ethylene and 1,2-Propylene Glycols

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Ethylene and 1,2-propylene glycols can be determined simultaneously by periodate oxidation to formaldehyde and acetaldehyde which are determined polarographically after a simple distillation. The method is applicable to the analysis of mixtures of formaldehyde and acetaldehyde as well as to the determination of other substances which form these aldehydes on treatment. The principal limitation of the method is that there must not be formed any other volatile substances polarographically reducible under the same conditions as the aldehydes.

ALTHOUGH several methods for the quantitative determination of glycols have been described in the literature, little attention has been given to their determination in mixtures of each other. Those methods that have been used involve tedious and time-consuming procedures. It seemed desirable, therefore, to obtain a simple and rapid method for quantitatively determining the amounts of ethylene and 1,2-propylene glycols in the presence of one another.

The method described in this paper consists essentially of oxidizing the glycol mixture with periodic acid to give formaldehyde and acetaldehyde according to the following equations:

$$CH_2OH.CH_2OH + HIO_4 \longrightarrow 2HCHO + HIO_3 + H_2O$$

$$CH_3.CHOH.CH_2OH + HIO_4 \longrightarrow HCHO + CH_3.CHO + HIO_3 + H_2O$$

The resulting aldehydes are then determined polarographically.

From an examination of the equations of the reaction, it is seen that the acetaldehyde content gives a measure of the propylene glycol concentration. The ethylene glycol can be estimated by deducting the formaldehyde produced by the oxidation of propylene glycol from the total amount of formaldehyde found in the mixture.

Johnson (*5*) determined 2,3-butylene glycol by oxidizing it with potassium periodate in an acid medium to give acetaldehyde. This was distilled into a standard bisulfite solution to form the bisulfite addition product, which was subsequently determined by titration of the bound bisulfite with standard iodine solution. Hoepe and Treadwell (*3*) described a method utilizing the Malaprade reaction for the determination of glycerol, ethylene glycol, and 1,2-propylene glycol in mixtures. The resulting formic acid, total aldehyde, and formaldehyde were determined titrimetrically on aliquots of the oxidized solution. Mixtures of formaldehyde and acetaldehyde were determined by Ionescu and Slusanschi (*4*) using dimedon (dimethyldihydroresorcinol) as a precipitant, and noting the length of time required for the crystals to appear.

Boyd and Bambach (*1*) described a polarographic method for the determination of formaldehyde as a step in the procedure for the determination of serine in protein hydrolyzates. The method consisted of treating the hydrolyzate with periodic acid, quantitatively separating the resulting formaldehyde by distillation, and determining it polarographically using 0.05 *N* potassium hydroxide in 0.1 *N* potassium chloride as the supporting electrolyte solution. The effect of the presence of other aldehydes such as acrolein, acetaldehyde, and propionaldehyde on the determination of formaldehyde was investigated by Whitnack and Moshier (*6*). They found that the most satisfactory results are obtained in 0.1 *N* lithium hydroxide containing 0.01 *N* lithium chloride at constant temperature and constant pH without the removal of dissolved oxygen.

EXPERIMENTAL

The procedure for the polarographic determination of formaldehyde in the presence of acetaldehyde as previously described by Whitnack and Moshier was followed in this study. A solution consisting of 0.1 *N* lithium hydroxide in 0.01 *N* lithium chloride was used as the supporting electrolyte medium. It was not necessary to add a maximum suppressor, inasmuch as no maximum was found to occur under the conditions used.

Preliminary studies revealed that the formaldehyde wave appeared at −1.45 to −1.58 volts (dropping mercury electrode *vs.* pool) and the acetaldehyde wave appeared at −1.65 to −1.85 volts under the conditions of pH, concentration range, and capillary used. In the actual determination, it was found necessary only to determine the galvanometer readings at a point before the appearance of the formaldehyde wave, −1.400 volts, at the height of the formaldehyde wave, −1.610 volts, and at the height of the acetaldehyde wave, −1.890 volts. These are the only points required for the quantitative determination of the aldehyde content of the mixture resulting from the oxidation of the glycols.

It was found that the height of the acetaldehyde wave decreased markedly (approximately 15% after 1 hour) when allowed to stand in contact with the alkaline electrolyte solution. The formaldehyde on the other hand was only slightly affected (approximately 5% decrease after 1 hour). For this reason, and also because of the volatilization of the acetaldehyde while in the polarographic cell, all determinations were made after standing in contact with the base solution in the cell for the same length of time (5 to 10 minutes).

MATERIALS AND APPARATUS

GLYCOLS. Pure ethylene glycol (Coleman and Bell Co.) and redistilled 1,2-propylene glycol (Eastman Kodak Co.) were used. The refractive indices and the specific gravities of the glycols checked the values reported in the literature.

ALDEHYDES. A stock standard solution of formaldehyde (0.795 mg. per ml.) was prepared from commercial formalin and standardized by the hydrogen peroxide method. A stock standard solution of acetaldehyde (0.470 mg. per ml.) was prepared by weighing a sample of pure acetaldehyde in an ampoule and diluting to a definite volume with distilled water; the acetaldehyde was purified by distillation through an efficient distillation column.

PERIODIC ACID. An approximately 0.5 *N* solution was prepared by dissolving 11 grams of periodic acid (The G. Frederick Smith Chemical Co.) in distilled water and diluting to 100 ml. The solution was stored in a dark glass-stoppered bottle.

BASE SOLUTION. This consisted of a 1 *N* lithium hydroxide solution in 0.1 *N* lithium chloride.

POLAROGRAPH. The Fisher Elecdropode was used in this work and all measurements were made at one tenth of the galvanometer sensitivity. The polarographic cell was a water-jacketed cell of about 35-ml. capacity connected in series through a small circulating pump with a constant-temperature bath thermostatically controlled at ±0.2° C.

DISTILLING APPARATUS. A simple distilling apparatus was used consisting of a semimicro 100-ml. Kjeldahl flask, a spray trap, a vertical condenser about 30 cm. in length, and a water bath for cooling the receiver.

PROCEDURE

PERIODIC ACID OXIDATION. An aqueous solution of the glycol mixture containing approximately 5 to 20 mg. of each of the glycols is pipetted into a 100-ml. Kjeldahl flask, and approximately 3 ml. of the periodic acid solution are added, with sufficient water to bring the total volume to about 60 ml. A few glass beads are introduced into the flask to prevent bumping.

The Kjeldahl flask is placed in an upright position and connected to the distilling unit. A deep receiver flask—e.g., a Pyrex test tube, 45 × 190 mm.—containing 75 ml. of distilled water is used to absorb the aldehydes and to cover the end of the condenser to a height of several centimeters. In order to minimize any loss of acetaldehyde due to volatilization, the receiver

is placed in an ice-water bath and kept cold throughout the course of the distillation.

The contents of the reaction flask are heated, gently at first, and the solution is distilled over at a rate of 3 to 4 ml. per minute until about 5 ml. remain in the Kjeldahl flask. Near the end of the distillation, the receiver flask is lowered so that the end of the condenser no longer extends below the surface of the liquid in the receiver. After the distillation is complete, the end of the condenser is rinsed with distilled water. The distillate is then quantitatively transferred to a 250-ml. volumetric flask, care being taken not to exceed a total volume of 225 ml. The aldehyde solution can be kept in this manner until ready for the polarographic measurements.

Polarographic Analysis. Immediately prior to the polarographic determination of the aldehydes, 25 ml. of the 1 N lithium hydroxide solution in 0.1 N lithium chloride are added to the distillate in the volumetric flask and the contents are diluted to the mark. In this way, the resulting solution has a concentration of 0.1 N lithium hydroxide in 0.01 N lithium chloride.

This practice seemed most advisable, since condensation of aldehydes is rapid in the presence of strong alkalies. By adding the base solution to the aldehyde mixture in the manner proposed, the time of contact of the aldehydes with the lithium hydroxide is reduced to a minimum.

The polarographic cell and electrodes are rinsed several times with the solution to be analyzed. A sample of the solution is then placed in the cell and allowed to come to constant temperature. After a definite length of time has elapsed from the moment of the addition of the supporting electrolyte solution, the galvanometer deflections at applied voltages of -1.890, -1.610, and -1.400 volts are read. The height of the acetaldehyde wave is the difference in the galvanometer reading between the first and second points; the height of the formaldehyde wave is the difference between the second and third points.

These wave heights are then compared to the values obtained from a 250-ml. solution prepared from the standard aldehyde solutions. The concentrations of formaldehyde and acetaldehyde in the latter solution should be approximately the same as those in the sample solution polarographed, though in the latter case it is not necessary to submit the aldehydes to a periodic acid oxidation or distillation, since there is no change in concentration as a result of such treatment.

CALCULATIONS

1,2-Propylene Glycol.

$$\text{Weight in sample, mg.} = W_p = \frac{H_a}{H_s} \times V \times C_s \times \frac{76}{44}$$

$$= \frac{1.727 \times V \times H_a \times C_s}{H_s}$$

where C_s = mg. of acetaldehyde per ml. of stock standard solution

V = volume in ml. of stock standard acetaldehyde solution taken

H_a = acetaldehyde wave height from periodic acid oxidation of glycol mixture

H_s = acetaldehyde wave height of standard

$$\text{Per cent by weight} = \frac{100\,W_p}{S}$$

where S = weight of sample in mg.

Ethylene Glycol.

$$\text{Weight in sample, mg.} = W_e = \frac{1.033 \times V \times H_f \times C_s'}{H_s'} - 0.408\,W_p$$

where W_p = mg. of 1,2-propylene glycol in sample

C_s' = mg. of formaldehyde per ml. of stock standard solution

V = volume in ml. of stock standard formaldehyde solution taken

H_f = formaldehyde wave height from periodic acid oxidation of glycol mixture

H_s' = formaldehyde wave length of standard

$$\text{Per cent by weight} = \frac{100\,W_e}{S}$$

where S = weight of sample in mg.

DISCUSSION

The values obtained at three different temperatures indicates that, within the range of 20° to 30° C., the height of the formaldehyde wave increases approximately 6.5% for each 1° C. rise in temperature. This is in agreement with the observation of Boyd and Bambach (*1*). The acetaldehyde wave height, however, increases only approximately 1.5 to 2.0% per degree rise in temperature, which is the same as that found by Elving and Rutner (*2*).

The data obtained in these studies indicate that, under the conditions used, there is a linear relationship between the height of the wave and the corresponding aldehyde content of the solution resulting from the action of periodic acid on the glycol mixture.

Using synthetic mixtures of the pure glycols an average precision and accuracy of better than 1.5% was obtained for propylene glycol and 2.2% for the ethylene glycol.

It is important that any material capable of being oxidized by periodic acid to give formaldehyde or acetaldehyde be absent. This includes such substances as α-amino alcohols, hydroxyamino acids (serine and threonine), and polyalcohols (glucose and other sugars). Monohydroxy alcohols, in general, such as methanol or ethanol do not affect the determination.

The polarographic method described need not necessarily be limited to the determination of mixtures of glycols but can also be successfully applied to the determination of formaldehyde and acetaldehyde in mixtures and to the determination of substances which form these aldehydes on oxidation—e.g., a mixture containing 1,2-propylene glycol and glycerol. The real limitation of the technique described—oxidation to formaldehyde and acetaldehyde, separation of the aldehydes, and polarographic measurement of the aldehyde content—is that there be absent and not produced any volatile substances polarographically reducible in the same potential range as the aldehydes under the conditions used.

Representative data on the analysis of mixtures of ethylene glycol and 1,2-propylene glycol in the manner described are given in Table I.

Table I. Simultaneous Determination of Ethylene Glycol and 1,2-Propylene Glycol in Synthetic Mixtures

Ethylene Glycol			Propylene Glycol		
Present	Found	Error	Present	Found	Error
Mg.	*Mg.*	%	*Mg.*	*Mg.*	%
4.31	4.28	−0.7	8.26	8.13	−1.6
4.31	4.53	+5.1	16.52	16.50	−0.1
4.31	4.34	+0.7	16.52	16.72	+1.3
8.62	8.95	+3.8	16.52	16.43	−0.2
8.62	8.55	−0.8	16.52	16.71	+1.1
8.62	8.25	−4.3	16.52	17.02	+2.8
10.94	11.02	+0.7	(15.16)	...	...
10.94	10.66	−2.5	15.16	15.38	+1.4
10.94	10.91	−0.3	15.16	15.52	+2.4
10.94	10.98	+0.4	15.16	15.26	+0.7
10.94	10.98	+0.4	15.16	15.18	+0.1
10.94	10.60	−3.1	22.74	22.55	−1.1
16.41	15.93	−2.9	15.16	14.91	−1.6
16.41	17.34	+5.7	22.74	23.02	+1.1
Av.		±2.2			±1.3

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Colorimetric Determination of Iron

In the Presence of Large Concentrations of Copper and Nickel

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The usefulness of disodium-1,2-dihydroxybenzene-3,5-disulfonate as a chromogenic reagent for the determination of iron has been extended. A technique has been applied which allows estimation of iron in the presence of large quantities of copper and nickel. As little as 0.025% iron in blue vitriol can be estimated without prior separations.

YOE and Jones (*2*) proposed the use of disodium-1,2-dihydroxybenzene-3,5-disulfonate (tiferron) as a colorimetric reagent for the estimation of ferric iron. They found that comparatively large concentrations of a number of diverse ions offer no interference, but that, in the presence of 1.0 p.p.m. of iron, the copper concentration must be less than 25.0 p.p.m.

In the writer's laboratory, iron determinations are made on a considerable number of liquors incident to the electrolytic refining of copper. These liquors contain comparatively small amounts of iron in the presence of sulfuric acid, arsenic, antimony, up to 18% copper, and up to 8% nickel. In addition, small percentages of iron are determined in blue vitriol samples.

A method which would permit iron estimations without prior separations of the copper and nickel would have obvious advantages. Silverthorn and Curtis (*1*) determined chromium in the presence of manganese which interferes at the wave length employed. They measured the total absorption caused by the manganese and chromium together and then corrected for the light absorbed by the manganese alone. Standard curves were used for this purpose.

It was the purpose of this investigation to attempt to devise a method for estimating iron in the presence of arsenic, antimony, nickel, and copper, using the technique of Silverthorn and Curtis as a basis and tiferron as the chromogenic reagent.

APPARATUS

All the optical measurements were made with a Coleman model 11 Universal spectrophotometer having a band width of 35 mμ. Cylindrical glass cells having an inside diameter of 16 mm. were employed.

SOLUTIONS

TIFERRON. This reagent was obtained from LaMotte Chemical Products Co. An aqueous solution containing 25 grams per liter was used.

BUFFER. This solution contained 20 grams of sodium hydrogen carbonate and 10 grams of sodium carbonate per liter of solution, twice the strength of the buffer used by Yoe and Jones (*2*). The stronger solution was used in order to obtain the proper pH with test aliquots containing sulfuric acid.

STANDARD IRON. Analytical grade iron wire was dissolved in dilute hydrochloric acid. The solution was oxidized with bromine water and the excess expelled by boiling. The solution was made up to 1 liter and used as a stock solution.

STANDARD NICKEL. A stock solution was made from reagent grade nickel sulfate crystals having an iron concentration of 0.0005%. Its exact strength was determined electrolytically.

STANDARD COPPER. A stock solution was made from reagent grade copper sulfate pentahydrate crystals. Exact strength was determined electrolytically. Crystals contained 0.00035% iron.

GENERAL PROCEDURE

Transfer a suitable aliquot to a 50-ml. volumetric flask and add enough tiferron solution to take care of all the copper, nickel, and iron present. Mix, dilute to the calibration mark with the buffer solution, and mix thoroughly. Transfer a portion of the solution to an absorption cell and measure the light transmittancy at 500 mμ, using water as the reference solution. Readings should be taken within 4 minutes following the addition of the buffer solution. The per cent iron is read from tables, prepared as described below.

The procedure presupposes some knowledge as to the approximate concentrations of copper and nickel in the sample. If the nickel content is not more than sixty times that of the iron, its effect may be neglected.

The minimum quantity of tiferron required for the test is determined by the amounts of iron, copper, and nickel present. Experiments have shown that in terms of dry tiferron, the respective requirements for 1 part of iron, 1 part of copper, and 1 part of nickel are 40, 12, and 1.25 parts. These quantities are somewhat in excess of minimum requirements. It has been found that an excess of the reagent has no effect in the presence of copper and iron but does influence the results to a negligible extent when nickel is present. A large excess of the reagent should be avoided because of its cost.

WAVE LENGTH–TRANSMITTANCY RELATIONS

The wave length–transmittancy characteristics for iron, copper, and nickel are shown in Figure 1. The respective concentrations used for these tests were 0.050, 15.0, and 49.0 mg. per 50 ml.

It will be noted that the shapes of the nickel and copper curves are similar and differ from that of the iron curve. The maximum absorption for iron corresponds to a wave length of about 490 mμ, which is close to the 500 established for the testing work. The absorption minima for copper and nickel are in the neighborhood of 550 mμ.

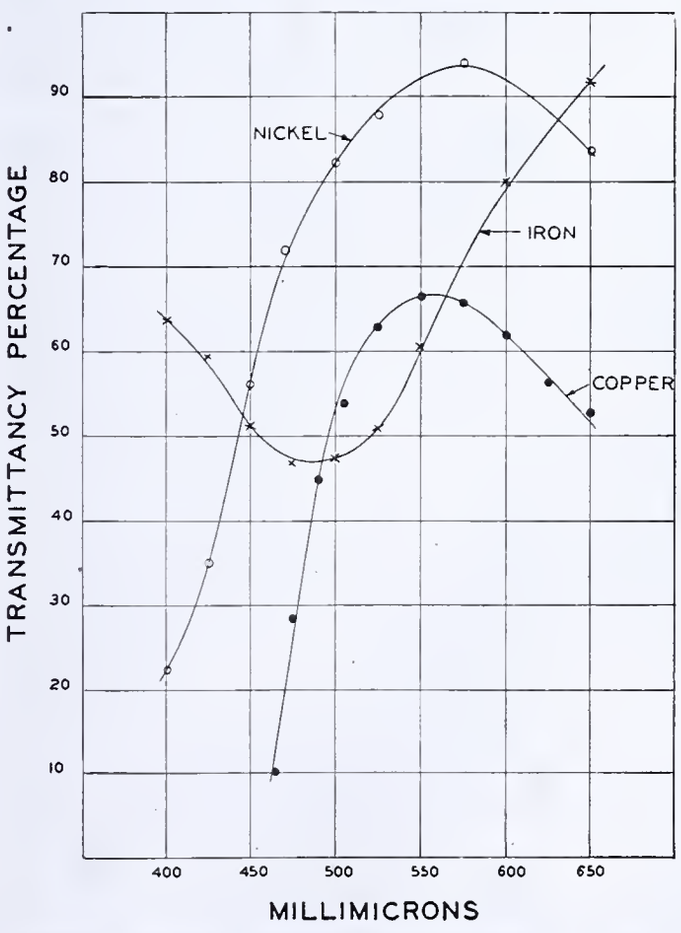

Figure 1. Transmittancy Curves for Nickel, Iron, and Copper

Table I. Aliquot Schedule

Type of Material	Initial Sample Weight	Dilution Volume	Volume of Aliquot	Volume of Tiferron	Use Schedule
	Grams	*Ml.*	*Ml.*	*Ml.*	
A liquor	5.0	200	2.0	12	A
B liquor	5.0	200	1.0	2	B
C liquor	5.0	200	1.0	2	B
D liquor	5.0	200	2.0	8	A
E liquor	5.0	200	1.0	2	B
Blue vitriol	25.0	500	2.0	14	C

Table II. Iron Schedule

% Transmittancy	% Iron		
	4% Cu	6% Cu	8% Cu
	Schedule A, A and D liquors		
50	0.148	0.136	0.122
49	0.154	0.142	0.128
48	0.160	0.148	0.134
47	0.165	0.153	0.139
	Schedule C, Blue Vitriol		
	24% Cu	25% Cu	26% Cu
25	0.045	0.040	0.035
24	0.050	0.045	0.040
23	0.056	0.051	0.046
22	0.062	0.057	0.052
21	0.069	0.064	0.059

CONCENTRATION-TRANSMITTANCY RELATIONS

The concentration–transmittancy relations were investigated for copper, nickel, and iron at 500 mμ (Figure 2). The procedure outlined above was followed, using varying quantities of the standard solutions.

The curves cut the vertical axis at a point representing 96.5% transmittancy instead of the expected 100%, because water, rather than a blank containing the buffer and tiferron, was used as the reference solution.

It will be seen that Beer's law holds for nickel and iron, while there is a considerable deviation corresponding to the lower concentrations of copper.

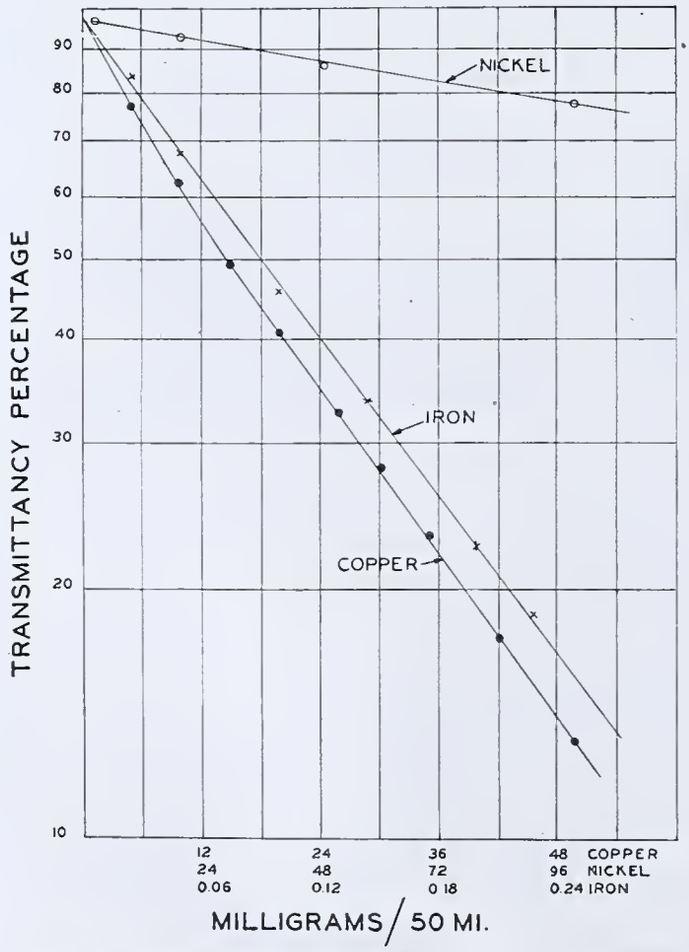

Figure 2. Transmittancy-Concentration Curves for Nickel, Iron, and Copper

The absorption by the nickel complex is comparatively low and its effect may be neglected with respect to lower concentrations.

At higher nickel concentrations, corrections should be applied for the nickel present. The method outlined below for copper can be used. Because of the increase in light absorption with time, readings should be taken within 4 minutes after addition of the buffer as recommended in the general procedure.

The colors developed by copper and iron are relatively stable, no material change in transmittancy taking place over the first half hour.

Comparatively high concentrations of arsenic and antimony, both trivalent and quinquevalent, showed no light absorption at 500 mμ. No further consideration has, therefore, been given these elements.

CORRECTION FOR COPPER PRESENT

The presence of copper in a sample will manifest itself by an increase in the light absorbed, resulting in an apparent increase in the concentration of iron. Readings must therefore be corrected for the copper present.

This may be done by referring to the copper curve in Figure 2 and noting the transmittancy corresponding to the known copper concentration. Reference is then made to the iron curve, and the iron concentration corresponding to this transmittancy is the correction for the copper present. This correction is subtracted from the total apparent iron equivalent to the actual instrument reading. The difference is equal to the true iron concentration.

An example will make this clear. Assume that an aliquot contains 12 mg. of copper and that the instrument reading is 40. Reference to the copper curve in Figure 2 shows that the percentage transmittancy corresponding to 12 mg. of copper is 56. Reference to the iron curve shows that 56% transmittancy is equivalent to 0.074 mg. of iron. The apparent iron equivalent to the actual instrument reading of 40% is 0.12 mg. The true iron content is then 0.12 − 0.074 or 0.046 mg.

As an aid in routine work, a table has been arranged to cover some of the samples tested in the author's laboratory showing the dilution, size of aliquot to be taken, and volume of tiferron to be used. In addition, tables have been assembled to show the concentration of iron corresponding to various instrument readings and corrected for a number of different copper concentrations. Abbreviated forms are represented by Tables I and II.

CHECKS ON KNOWN MIXTURES

Some determinations were made on "synthetic" mixtures containing varying quantities of copper, nickel, and iron (Table III).

The results of determinations made on some production materials whose iron contents had previously been determined gravimetrically are shown in Table IV.

In making these determinations, a quantity of the sample was transferred to a beaker, diluted with water, and 5 ml. of 1 to 4

Table III. Iron Checks on Synthetic Mixtures

Composition of Mixture			Fe Found
Cu	Ni	Fe	
	Mg./50 ml.		*Mg./50 ml.*
6	1.44	0.060	0.058
8	1.44	0.030	0.031
10	0.028	0.010	0.018
10	0.028	0.030	0.040
15	2.80	0.050	0.052
20	0.056	0.020	0.025
20	0.056	0.030	0.034
20	0.056	0.060	0.063
30	0.112	0.010	0.018
30	0.112	0.030	0.040
30	0.140	0.070	0.068
30	0.140	0.090	0.090
30	0.140	0.100	0.100
30	1.40	0.070	0.071
30[a]	14.00	0.070	0.075
30[a]	49.00	0.070	0.070
30[a]	98.00	0.070	0.070

[a] Corrected for nickel present.

Table IV. Iron Checks on Materials in Process

Type of Material	Approximate Percentage				Percentage of Fe	
	As	Sb	Cu	Ni	Gravimetric	Colorimetric
H-3 liquor	0.2	0.03	2.70	0.70	0.024	0.023
H-3 liquor	0.2	0.03	2.67	0.74	0.025	0.025
H-3 liquor	0.2	0.03	2.70	0.72	0.028	0.031
H-4 liquor	0.2	0.03	1.75	0.82	0.025	0.025
H-4 liquor	0.2	0.03	1.71	0.85	0.028	0.027
H-4 liquor	0.2	0.03	1.95	0.82	0.038	0.034
H-5 liquor	0.2	0.03	2.64	0.65	0.021	0.019
H-5 liquor	0.2	0.03	2.92	0.67	0.022	0.020
O liquor	0.6	..	15.20	0.40	0.054	0.050
A liquor	1.2	..	15.70	1.70	0.230	0.215
E liquor	2.8	..	3.80	4.50	0.416	0.365
G liquor	0.3	0.08	3.00	..	0.024	0.023
Blue vitriol	...	..	25.1	..	0.032	0.025
	...	..	25.1	..	0.025	0.020
	...	..	25.1	..	0.026	0.024
	...	..	25.1	..	0.026	0.027
	...	..	25.1	..	0.070	0.063
	...	..	25.1	..	0.086	0.080

sulfuric acid were added to prevent the precipitation of arsenic and antimony. A few milliliters of saturated bromine water were then added and the excess was expelled by boiling. The solution was cooled, and if the acidity was initially high it was neutralized by means of ammonium hydroxide, and a slight excess of 1 to 4 sulfuric acid added. The test was again cooled, transferred to a volumetric flask, diluted to the mark, and thoroughly mixed. An aliquot was transferred to a 50-ml. volumetric flask and the general procedure outlined above was followed.

DISCUSSION

Iron may be determined in the presence of large quantities of copper and nickel with excellent accuracy, provided the iron concentration is not less than 0.05 mg. per 50 ml. Below this concentration, the expected error is somewhat greater but will still be sufficiently small for many purposes.

The iron was accurately determined in a "synthetic" mixture containing 30 mg. of copper, 98 mg. of nickel, and 0.070 mg. of iron per 50 ml. In this case it was necessary to correct for both the copper and nickel present.

The maximum concentration of nickel which may be present in a mixture is probably much higher than the maximum reported on in this investigation. In such cases, it would be necessary to take transmittancy readings very quickly following addition of the buffer solution.

It is believed that the utility of many of the published spectrophotometric analytical methods can be extended materially by the use of corrected tables similar to those described in this paper. The author is now applying this technique to the estimation of small concentrations of nickel in samples of the type shown in Table IV and expects to publish the findings at a later date.

ACKNOWLEDGMENT

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Destruction of Organic Matter in Blood Fibrin and Chromacized Medical Catgut by Wet Oxidation

Determination of Iron in Blood and of Chromium in Sutures

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THE determination of the mineral components of blood and the evaluation of chromium in chromacized medical catgut sutures are generally carried out starting with a dry-ashing procedure. This operation is time-consuming. Subsequent to dry-ashing at elevated temperatures the residue may require a fusion process to redissolve the ash. For these reasons a wet-ashing procedure is recommended and herein described.

The literature on the use of mixtures of nitric and perchloric acids in the wet-oxidation of organic matter preparatory to the determination of sulfur, phosphorus, and arsenic as well as the metallic components iron, chromium, aluminum, calcium, magnesium, sodium, and potassium has been previously surveyed (*4*, *7*). The important applications are the determination of sulfur in coal (*9*), chromium in leather (*3*), iron in leather (*6*), calcium, phosphorus, and the alkali metals in plant materials (*1*), sulfur in organic compounds (*4*), and iron in wine or beer (*7*).

CHEMICAL ASPECTS OF WET-OXIDATION ACID MIXTURES CONTAINING PERCHLORIC ACID

Because perchloric acid is known, when hot and concentrated, to exert a powerful oxidizing effect in contact with organic matter, its use for wet-ashing purposes is retarded by unwarranted apprehension of violent reactions. When properly used for wet-mineralization of samples composed preponderantly of organic matter, perchloric acid is entirely dependable. Samples of heterocyclic ring nitrogen compounds such as occur in coal which are oxidized with great difficulty by hot concentrated sulfuric acid, as in the Kjeldahl reaction, are readily and rapidly oxidized by mixtures of nitric acid and perchloric acid; vanadium must be used as catalyst to speed the oxidation to a time limit of 15 minutes (*9*).

The use of mixtures of concentrated nitric and perchloric acids as oxidants for organic materials of a wide variety of sources gives smooth, easily controlled, suitably rapid reactions because the mixture exerts a gradually increasing oxidation potential. The addition of concentrated nitric acid to concentrated (72%) perchloric acid dilutes the latter acid, which lowers its oxidation potential. At the same time the concentration of nitric acid, owing to its partial dehydration by the perchloric acid, increases its strength and oxidation potential. The mixed acids may be applied at ordinary temperatures and their oxidation effects gradually increased by elevating the temperature. The more easily oxidized portions of the organic matter are first attacked. As the temperature is raised the nitric acid excess is finally distilled off and the diluted perchloric acid begins to concentrate, and finally at 200° C. attains a constant boiling mixture (72% perchloric acid). During the course of this process its oxidation potential increases and the organic matter present which has escaped the nitric acid attack is gradually oxidized by the perchloric acid. The more easily oxidized organic material is destroyed by hot 60% perchloric acid and the most difficultly destroyed organic matter is finally oxidized by the boiling 72% perchloric acid, quietly and efficiently.

The most outstanding property of a mineralization reaction following mixed nitric-perchloric acid digestion at gradually elevated temperature is the fact that the organic matter is destroyed without accompanying carbonization, which is so pre-

dominant in such reactions as a Kjeldahl digestion. Most, if not all of the carbonaceous material is volatilized as gaseous products. With some types of organic material the reacting solution remains clear throughout the entire oxidation reaction. In other cases the solution acquires a chocolate-brown coloration due to degradation products of the decomposition of organic matter. In case any carbonaceous material is formed, the conditions are indicated as unsatisfactory and the proportion of mixed acids employed should be altered, the temperature relations changed, or additional reagent added to the reaction mixture—for example, sulfuric acid.

INFLUENCE OF SULFURIC ACID ADDITIONS TO OXIDATION MIXTURE

If the organic matter present affords smooth oxidation with extreme difficulty, the oxidation potential of perchloric acid may be very markedly increased by the addition of sulfuric acid. In this case the final state of oxidation is brought about through the the dehydration of perchloric acid by the sulfuric acid added and by the higher temperature produced in the latter stages of the reaction. The effect of the nitric acid is accomplished and the excess is boiled off at 140° to 150° C. The oxidation by perchloric acid begins at 150° to 160° C. as a concentration of the perchloric acid, due to the presence of sulfuric acid, does not stop at 72% strength, but may increase to 85% or even higher, depending upon the proportion of sulfuric acid present. Under these conditions the oxidation potential of the perchloric acid due to dehydration is gradually increased to a degree favorable to oxidations of the most resistant type. Use of sulfuric acid as a reaction component precludes, of course, the determination of sulfur and causes complications in presence of calcium salts.

INFLUENCE OF CATALYSTS

Just as in the Kjeldahl digestion, the oxidation time of which is much diminished in the presence of copper, mercury, selenium, etc., the presence of catalysts likewise shortens the reaction time with perchloric acid oxidation. Two such catalysts are chromium and vanadium. Here the mechanism of the reaction depends upon the fact that hot concentrated perchloric acid oxidizes chromium and vanadium to chromic and vanadic acids. These in turn are reduced by any traces of organic matter persisting and the process is repeated. When all organic matter is destroyed the perchloric acid oxidizes the chromium and vanadium to chromic and vanadic acids, which color the hot acid orange, and the chromic acid forms reddish orange crystals in the cooled 72% perchloric in which it is quantitatively insoluble. A few milligrams of either chromium or vanadium are sufficient for considerable diminution of the time required for the mineralization and the oxidation of a given sample of organic matter. Often their use causes a too energetic effect.

An estimate only can be made of the oxidation potential acquired by the gradual elevation of the temperature of the reaction mixture of nitric, perchloric, and sulfuric acids together, especially with the added influence of catalysts such as chromium and vanadium, or in some cases cerium. The temperature range covered is from room temperature, with gradually increasing increments, through the boiling range of nitric acid, perchloric acid, and finally sulfuric acid, at approximately 360° C. The estimated oxidation potential thus attained covers the range of 1 to 2 volts, as indicated by the ease with which chromium and cerium are oxidized to their highest stages of valence by such mixtures. The gradual increase in potential in ever-increasing increments is analogous to the playing of a chromatic scale on the piano keyboard from the lower pitched notes of the bass to the higher pitched notes of the soprano. With studied application of such oxidizing conditions to a given type of organic matter, no violent reactions are probable and the operation as a whole becomes perfectly reliable. Thousands of such reactions for the destruction of organic matter, in accordance with the literature references previously cited, are daily routine control procedures. For new applications, the analyst who prefers dry-ashing procedures, because of apprehension concerning hazardous application of the nitric-perchloric-sulfuric acid wet-ashing procedure, is depriving himself of one of the most reliable, time-saving, and precise procedures. Conditions, once established for the proper application of this procedure to a specific case, bring into play easily recognized superior advantages. The present discussion demonstrates these facts as applied to the mineralization of blood fibrin and chromacized catgut sutures.

APPARATUS EMPLOYED

An electrically heated hot plate with inset rheostat for variable heat control, such as is always available from reagent supply houses, or a modified (*8*) Rogers ring burner and Nichrome wire gauze can be used, and intermediate heat intensities other than those provided by the device itself can be arranged by the use of heavy asbestos paper in one-, two-, or three-layer portions. The actual heat attained under given conditions should be determined by the use of the acids employed without sample present. In general, a beaker containing concentrated sulfuric acid and inserted thermometer serves as an adequate test. A maximum temperature of 210° to 225° C. was found suitable for the two types of samples under discussion. The use of the Rogers ring burner as a substitute for electrical heating provides very suitable variable heat adjustment.

Vycor glass Erlenmeyer flasks, 500-ml. capacity, should be employed for safety from cracking or fracture due to sudden heat gradients. Their use avoids all danger that a flask containing hot oxidizing acids may crack or open at the bottom, causing damage through fire to wooden table tops or fume hood floors. These flasks entail some additional expense in first costs, but they are more than justified by the increased facility with which they may be rapidly cooled and heated. For the determination of chromium in sutures they are practically indispensable, since the flask and contents must be chilled instantly from 200° C. to ice bath temperatures to prevent the hydrogen peroxide error (*4*).

An efficient fume hood with forced draft ventilation is essential if a large number of operations are to be carried out at one time. The excess acids must be volatilized. The organic degradation products resulting from the oxidation by this "cracking" process are acrid and disagreeable. If such a fume hood is not available, a perfect substitute has been described (*5*).

DESTRUCTION OF ORGANIC MATTER IN BLOOD FIBRIN

The oxidation is complete, rapid, and smooth running if perchloric acid alone is employed. The reaction is begun after addition of 60% perchloric acid by placing the acid-treated sample directly on the hot plate capable of elevating the temperature eventually to 210° to 225° C. The blood fibrin is at the start dissolved in the perchloric acid, giving a deep purple product. After 3 minutes the color becomes chocolate brown, accompanied by noticeable carbonization. With 10 minutes' treatment there is a generous foaming reaction, as evolved gases

Table I. Destruction of Organic Matter in Blood Fibrin Using Nitric and Perchloric Acids

Determination	Blood Fibrin	72% $HClO_4$	67% HNO_3	Time Completion, HNO_3 Reaction	Total Reaction Time
	Grams	*Ml.*	*Ml.*	*Min.*	*Min.*
1	3	10	10	7	13
2	4	10	10	5	13
3	5	12.5	17.5	7	14
4	6	15	20	5	20
5	7	15	20	6	20
6	8	15	25	7	33
7	9	15	25	7	16
8	10	17.5	30	8	15
		With Addition of 25 Mg. of CrO_3			
9	5	15	15	5	28
		With Addition of 0.001 Gram of Ammonium Vanadate			
10	10	17.5 60% $HClO_4$	30	6	13
11	1	15	Absent	..	18
12	2	15	Absent	..	15
13	3	21	Absent	..	13
14	4	21	Absent	..	18
15	4	25	Absent	..	21
16	5	30	Absent 2 mg. $(NH4)_4VO_4$	..	19

re given off together with fumes of perchloric acid and the reaction mixture rapidly turns light brown. In 5 additional minutes' heating time the reaction is complete, as shown by the absence of any color except that from a little chlorine resulting from a light decomposition of the hot concentrated acid. The addition of 2 mg. of ammonium vanadate as catalyst was of no appreciable advantage. The volume of 60% perchloric acid used should be 5 to 6 ml. per gram of blood fibrin with a minimum of 10 to 15 ml. to provide enough material to cover the bottom of the 500-ml. Vycor flask. Results obtained are given in Table I, determinations 11 to 16.

The time rate of oxidation of blood fibrin is increased by the use of 67% nitric and 72% perchloric acids. No carbonization of the organic matter occurs. Reaction results show a vigorous oxidizing action due to nitric acid accompanied by a rapid evolution of nitric oxide and gaseous decomposition products. During this stage the frothing may amount to a 2.5- to 5-cm. (1- to 2-inch) thick layer. After 3 to 4 minutes the nitric acid reaction is complete and after an additional 4 to 5 minutes the excess nitric acid has been boiled off. The solution then rapidly turns chocolate brown in color, a 1.25-cm. (0.5-inch) foam coating appears, and fumes of the decomposition products of perchloric acid form. The color of the solution rapidly becomes lighter, carbon dioxide is evolved, and the reaction is complete in 15 to 18 minutes if generous portions of nitric acid are employed. Chromic acid as catalyst brings no time advantage and as little as 0.5 mg. of vanadium causes the reaction mixture to catch fire (Table I, determinations 10 and 9). Osmic acid is of no advantage in speeding up the reaction.

DETERMINATION OF IRON IN BLOOD FIBRIN

For determining the effective recovery of iron in blood, samples of 5 grams each are oxidized, as previously described, and the acid-soluble residue containing excess perchloric acid is transferred from the 500-ml. Vycor flask to a 150-ml. Pyrex beaker. The samples are then evaporated on a hot plate with the beakers covered and the cover glass elevated, using twin watch glass supports (*2*), until the perchloric acid is completely removed. If a trace of brown appears in the otherwise white residue in spots, 0.5 ml. more of 72% perchloric acid is added and reheated until the excess is volatilized and the last trace of oxidizable material is evolved. Finally the contents of the beaker are cooled, dissolved in water, and transferred to a 250-ml. graduated flask. It is not as a rule necessary to filter because of the presence of a little dehydrated silica.

Now 1 ml. of a 10% hydroxylamine hydrochloride solution is added, and a small piece of Congo red paper which will turn dark blue because of the acid present. A solution of dilute aqueous ammonia is added, a few drops at a time, until the Congo red paper just turns pink (pH 4 to 4.5), followed by addition of 5 ml. of a saturated aqueous solution of 1,10-phenanthroline. The red color of 1,10-phenanthroline ferrous iron immediately appears. The solution is then diluted to the mark in the graduated flask, and mixed. The color intensity is determined using a Cenco Photelometer or its equivalent and a 1-cm. cell with a green filter. From a calibration chart plotted with log transmittancy on the vertical axis and milligrams of iron on the horizontal, the amount of iron present can be rapidly determined. If the samples are not filtered before color production, the color comparisons must be made immediately after color formation.

To determine the accuracy with which iron is retained by the mixed acid mineralization of the product, samples of blood fibrin were made ready and known amounts of iron added before oxidation. As a result of a group of analyses of the unfortified blood fibrin the iron present was found to be 70 parts per million; 200 micrograms of iron added to fortify the blood samples could be then recovered within an accuracy of 5 to 6 micrograms.

Since sulfur is present in blood fibrin, the treatment described is capable of oxidizing it to sulfate. Because of the presence of sulfur, special treatment is required if sodium and potassium are to be determined in the residue from oxidation of organic matter.

DESTRUCTION OF ORGANIC MATTER IN CHROMACIZED CATGUT

Catgut sutures of varying degrees of chromacization were cut into 6-mm. lengths and dried for 8 to 12 hours in an electric oven at 80° C. and stored in glass weighing bottles in preparation for mineralization and determination of chromium.

Weighed samples of 1 to 2 grams were transferred to 500-ml. Vycor Erlenmeyer flasks and treated with varying proportions of 72% perchloric acid and 67% nitric acid. The most satisfactory mixture was found to be 20 ml. of perchloric to 6.5 to 7.0 ml. of nitric acid.

The sample, in a short time after heating begins, is completely dissolved. In 5 or 6 minutes the nitric acid reaction accompanied by the production of brown fumes is over and the excess nitric acid is volatilized. During this process it would appear that nitration products of the organic matter are formed. During and at the end of the nitric acid reaction the solution is green in color. As the reaction mixture rises from 140° through 160° to 180° a vigorous reaction takes place, with the copious formation of gaseous products of reaction, but little or no foaming. The solution may or may not turn light brown in color, depending upon the rate of temperature elevation. In 8 to 13 minutes the decomposition of organic matter is complete, as shown by the fact that the color of chromic acid appears.

The sample is kept on the hot plate for 2 to 3 minutes after the appearance of the chromic acid color at not more than 200° C. and is quickly removed from the hot plate and plunged into an ice bath with a swirling motion during 5 seconds. It is then quickly diluted by the addition of 60 to 70 ml. of cold water, and the refluxing still head is rinsed into the flask and removed. A boiling chip of Filtercel is added and the solution boiled 2 to 3 minutes to remove the small amount of chlorine formed during the oxidation reaction. The solution is then diluted to 200 ml. with cold distilled water, a drop of 0.025 *M* ferroin indicator is added, and the solution is titrated using 0.033 *N* ferrous sulfate to the first production of a pink cast to the solution from the first drop excess of reducing agent. One ml. of 0.033 *N* ferrous sulfate solution corresponds to 1.1112 mg. of CrO_3.

The results from a series of analyses are given in Table II.

Table II. Mineralization of Chromacized Catgut and Determination of Chromic Oxide

(20 ml. of 72% $HClO_4$ and 6.5 ml. of 67% HNO_3 per sample. Time for HNO_3 reaction, 5 to 6 minutes. Hot plate temperature, 210° to 225° C. during oxidation of sample and 200° C. during oxidation of chromium)

Sample No.	Weight	Reaction Time	0.033 *N* Fe^{++} Required	CrO_3 Present
	Grams	*Min.*	*Ml.*	%
1	2	16	25.15	1.40
	2	13	25.30	1.41
	2	9	25.04	1.39
				Av. 1.40 ± 0.01
2	2	11	31.55	1.75
	2	9	31.42	1.75
				Av. 1.75
3	2	11	31.90	1.77
	2	10	30.60	1.70
				Av. 1.74 ± 0.04
4	2	11	31.70	1.76
	2	13	31.60	1.76
				Av. 1.76
5	2	10	41.32	2.30
	2	10	41.60	2.31
	2	9	41.87	2.33
	2	10	41.08	2.28
				Av. 2.30 ± 0.03
6	1.002	15	14.65	1.62
	1.006	15	14.72	1.63
	1.012	13	14.51	1.61
				Av. 1.61 ± 0.01
7	1.0037	15	16.03	1.78
	1.0017	14	16.19	1.80
				Av. 1.79 ± 0.01

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Analysis of Mixtures of Meta- and Para-Cresols and Their Butylated Products

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Data are presented for the analysis, by freezing point and cloud point determinations, of binary cresol and butylated cresol mixtures such as are encountered in the butylation-fractionation-debutylation method of separating *m*- and *p*-cresols. The procedures are simple and rapid, and yield results which are readily duplicated. A cloud point method for determining small amounts of water in glycols and glycerol is suggested.

FROM their mixtures *m*- and *p*-cresols are separated by a method involving alkylation with isobutylene, separation by fractional distillation of the tertiary butyl derivatives so produced, and dealkylation of the individual di-*tert*-butylcresols to give the pure parent cresols (*3*). In the commercial operation (*4*) it is important that reliable and quick methods be available for analyzing the various distillates handled. The determination of freezing points is a convenient method of analysis. A measurement of the critical solution temperature of the phenolic mixture in diethylene glycol and other solvents offers another rapid and accurate test for this purpose.

MATERIALS

The *m*- and *p*-cresols employed in securing the data reported herein were Eastman practical grade chemicals which were subjected to a purification sequence comprising fractional distillation under reduced pressure and at about 15 to 1 reflux ratio through a 20-plate packed glass column, collecting only the middle portion which came over within a 1° C. temperature range. This material was melted, allowed to cool, and seeded. When the crystallization was about half complete, the crystals formed were separated from the mother liquor and distilled through the 15-plate column, again collecting only a heart cut. The tertiary butylated derivatives of these cresols were prepared by alkylation with isobutylene (*3*) and purified by fractionation-recrystallization-fractionation treatments. Triisobutylene was made by the action of 63% sulfuric acid on Eastman *tert*-butyl alcohol and purified by means of an alkali wash, a rough distillation through a Hempel column, and two fractional distillations through the 20-plate column. The cresols, their butylated derivatives, and the triisobutylene were believed to be of at least 99.5% purity. Their properties are listed in Table I.

Table I. Properties

Name of Compound	Boiling Point at 20 Mm. °C.	Freezing Point °C.
m-Cresol	...	11.5[a]
p-Cresol	...	34.7[a]
Mono-*tert*-butyl-*m*-cresol	...	21.3
2-*tert*-Butyl-4-methylphenol	126.5	51.7
4,6-Di-*tert*-butyl-3-methylphenol	167.0	62.1
2,6-Di-*tert*-butyl-4-methylphenol	147.0	69.7[b]
Triisobutylene	174.9–5.3[c]	...

[a] W. Seaman, Laboratory for Analytical Research, Calco Chemical Division, American Cyanamid Co., Bound Brook, N. J. (private communication), reports highly purified *m*- and *p*-cresols to freeze at 11.65° and 34.63° C., respectively.
[b] Melting point.
[c] At 745.7 mm.

Table II. Freezing Points of Mixtures of *m*- and *p*-Cresols[a]

p-Cresol %	*m*-Cresol %	Freezing Point ° C.	*p*-Cresol %	*m*-Cresol %	Freezing Point ° C.
0.0	100.0	11.5	44.5	55.5	5.5
2.6	97.4	9.6	52.7	47.3	2.1
6.5	93.5	6.6	59.0	41.0	−0.5
9.9	90.1	3.9	60.9	39.1	−0.4
12.2	87.8	2.1	65.2	34.8	4.0
13.1	86.9	2.5	72.1	27.9	10.7
18.2	81.8	5.5	80.1	19.9	18.6
23.8	76.2	8.0	88.2	11.8	25.5
30.1	69.9	8.1	95.1	4.9	31.2
31.5	68.5	8.4	100.0	0.0	34.7
35.1	64.9	7.6			

[a] Freezing point data on *m*- and *p*-cresol mixtures have been reported by Desseigne (*1*), who used cresols freezing at 10.1° and 34.5° C., respectively.

FREEZING POINTS

METHOD. A sample of 5 to 10 grams of the cresol mixture was placed in an unsilvered evacuated Dewar test tube (16×150 mm., inside dimensions) equipped with a glass loop stirrer and a calibrated thermometer, readable to 0.1° C., held in place by means of a cork stopper (an Anschütz thermometer was found very suitable for this work). When the material was a solid, the tube was first heated to melt the sample. After a preliminary trial, the Dewar test tube was placed in a water bath held at a temperature about 2° below the indicated freezing point. The

Table III. Freezing Points of Mixtures of *m*-Cresol and Mono-*tert*-butyl-*m*-cresol

m-Cresol in Mono-*tert*-butyl-*m*-cresol			Mono-*tert*-butyl-*m*-cresol in *m*-Cresol		
m-Cresol %	Mono-*tert*-butyl-*m*-cresol %	Freezing point ° C.	Mono-*tert*-butyl-*m*-cresol %	*m*-Cresol %	Freezing point ° C.
0.0	100.0	21.3	0.0	100.0	11.5
1.0	99.0	20.4	2.4	97.6	10.2
3.0	97.0	18.8	4.4	95.6	9.2
5.4	94.6	16.9	7.0	93.0	7.9
7.9	92.1	14.9	10.0	90.0	6.4
10.7	89.3	12.7	13.1	86.9	4.7
13.8	86.2	10.2	16.1	83.9	3.1
17.1	82.9	7.6	19.0	81.0	1.3
20.0	80.0	5.2	22.2	77.8	−0.3
21.4	78.6	3.6			

Table IV. Freezing Points of Mixtures of Mono-*tert*-butyl-*m*-cresol and 2-*tert*-Butyl-4-methylphenol

Mono-*tert*-butyl-*m*-cresol in 2-*tert*-Butyl-4-methylphenol			2-*tert*-Butyl-4-methylphenol in Mono-*tert*-butyl-*m*-cresol		
Mono-*tert*-butyl-*m*-cresol %	2-*tert*-Butyl-4-methylphenol %	Freezing point ° C.	2-*tert*-Butyl-4-methylphenol %	Mono-*tert*-butyl-*m*-cresol %	Freezing point ° C.
0.0	100.0	51.7	0.0	100.0	21.3
2.2	97.8	50.5	2.0	98.0	20.2
4.9	95.1	48.9	5.0	95.0	18.5
10.5	89.5	45.8	10.0	90.0	15.5
16.5	83.5	42.0	16.0	84.0	11.9
22.7	77.3	38.3	21.7	78.3	6.4
25.7	74.3	36.0	27.3	72.7	0.0
32.6	67.4	31.0	32.4	67.6	−3.2
36.0	64.0	29.2	34.8	65.2	−4.8
39.3	60.7	27.3			

Table V. Freezing Points of Mixtures of Mono-*tert*-butyl-*m*-cresol and 4,6-Di-*tert*-butyl-3-methylphenol

Mono-*tert*-butyl-*m*-cresol in 4,6-Di-*tert*-butyl-3-methylphenol			4,6-Di-*tert*-butyl-3-methylphenol in Mono-*tert*-butyl-*m*-cresol		
Mono-*tert*-butyl-*m*-cresol %	4,6-Di-*tert*-butyl-3-methylphenol %	Freezing point ° C.	4,6-Di-*tert*-butyl-3-methylphenol %	Mono-*tert*-butyl-*m*-cresol %	Freezing point ° C.
0.0	100.0	62.1	0.0	100.0	21.3
1.3	98.7	61.0	1.2	98.8	20.6
3.4	96.6	59.0	2.4	97.6	20.0
5.4	94.6	56.9	4.4	95.6	16.8
7.3	92.7	54.7	6.9	93.1	17.4
9.4	90.6	52.2	9.2	90.8	16.0
11.7	88.3	49.7	10.7	89.3	15.0
15.0	85.0	44.9	12.2	87.8	14.1
18.5	81.5	40.4	14.8	85.2	12.4

Table VI. Freezing Points of Mixtures of Triisobutylene and *m*-Cresol

Triisobutylene in *m*-cresol			Triisobutylene in *m*-cresol		
Triisobutylene %	*m*-Cresol %	Freezing Point ° C.	Triisobutylene %	*m*-Cresol %	Freezing Point ° C.
0.0	100.0	11.5	14.1	85.9	6.8
2.6	97.4	10.3	18.1	81.9	6.0
5.2	94.8	9.2	22.4	77.6	5.6
8.7	91.3	8.1	24.4	75.6	5.4
10.7	89.3	7.6			

Table VII. Critical Solution Temperatures

Weight Percentage of 2,6-Di-*tert*-butyl-4-methylphenol	Weight Percentage of Diethylene Glycol	Cloud Point, ° C.
75.0	25.0	181.2
58.2	41.8	190.0
49.1	50.9	190.2
47.4	52.6	190.1
37.5	62.5	189.0
27.0	73.0	181.7

Table VIII. Critical Solution Temperatures

Weight Percentage of 4,6-Di-*tert*-butyl-3-methylphenol in 2,6-Di-*tert*-butyl-4-methylphenol	Cloud Point, ° C. (Weight Ratio of Phenolic Mixture to Diethylene Glycol = 1.0 to 1.0)
0.00	190.2
1.45	188.5
3.42	185.4
5.76	181.5
8.79	176.0

Table IX. Critical Solution Temperatures

Weight Percentage of Mono-*tert*-butyl-*m*-cresol in 2,6-Di-*tert*-butyl-4-methylphenol	Cloud Point, ° C. (Weight Ratio of Phenolic Mixture to Diethylene Glycol = 1.0 to 1.0)
0.00	190.2
3.50	184.0
6.00	179.3
8.75	173.5

Table X. Critical Solution Temperatures

Weight Percentage of 2-*tert*-Buyl-4-methylphenol in 2,6-Di-*tert*-butyl-4-methylphenol	Cloud Point, ° C. (Weight Ratio of Phenolic Mixture to Diethylene Glycol = 1.0 to 1.0)
0.00	190.2
2.42	185.3
5.24	179.3
6.87	175.7
8.69	171.0
9.10	169.6

Table XI. Critical Solution Temperatures

Weight Percentage of 4,6-Di-*tert*-butyl-3-methylphenol	Weight Percentage of Blend (20% Glycerol and 80% Ethylene Glycol, by Volume)	Cloud Point ° C.
32.8	67.2	174.4
39.2	60.8	176.0
49.0	51.0	179.2
50.0	50.0	178.9
52.6	47.4	179.2
57.0	43.0	179.0
65.7	34.3	173.5

Table XII. Critical Solution Temperatures

Weight Percentage of 2,6-Di-*tert*-butyl-4-methylphenol in 4,6-Di-*tert*-butyl-3-methylphenol	Cloud Point, ° C. (Weight Ratio of Phenolic Mixture to Glycerol–Ethylene Glycol Blend = 1.0 to 1.0)
0.00	178.7
1.08	181.9
3.98	189.3
8.68	199.2

Table XIII. Effect of Water on Cloud Points of Alkylphenol Mixtures in Diethylene Glycol or in a Glycerol–Ethylene Glycol Blend

Water Added to Solvent	Cloud Point Rise: Equiweight mixture of (*i*) in (*j*)	Cloud Point Rise: Equiweight mixture of (*k*) in (*l*)
%	°C.	°C.
0.0	0.0	0.0
0.5	2.7	2.9
1.0	5.2	4.0[a]
1.5	6.9[a]	...

[a] Erratic results obtained caused by loss of water from solvent by vaporization.
(*i*) = mixture of 4% 4,6-di-*tert*-butyl-3-methylphenol and 96% 2,6-di-*tert*-butyl-4-methylphenol.
(*j*) = diethylene glycol (sp. gr. 1.1135/80° F.).
(*k*) = mixture of 4% 2,6-di-*tert*-butyl-4-methylphenol and 96% 4,6-di-*tert*-butyl-3-methylphenol.
(*l*) = blend comprising 20–80 volume mixture of glycerol and ethylene glycol.

ımple was then stirred until it was supercooled about 1° C. ıd, if necessary, seeded to bring about crystallization. After ystallization had started, the stirring was intermittent, about 5 to 1 minute between sets of 2 or 3 strokes of the stirrer. nder these conditions the temperature was found to rise to a ıaximum, where it remained until the greater part of the ma-ɛrial froze. The maximum value was taken as the freezing ɔint temperature of the sample.

The data obtained, given in Tables II to VI, are for the full ınge of *m*- and *p*-cresol mixtures, and for the particular distillate ɔmpositions handled in practice.

CRITICAL SOLUTION TEMPERATURES

The observation that mono- and di-*tert*-butyl-*m*-cresols and ıono-*tert*-butyl-*p*-cresol effect a pronounced lowering of the criti-ıl solution temperature of 2,6-di-*tert*-butyl-4-methylphenol with iethylene glycol, and that 2,6-di-*tert*-butyl-4-methylphenol ap-reciably raises that of 4,6-di-*tert*-butyl-3-methylphenol with a 0–80 volume mixture of glycerol–ethylene glycol was used as a asis for a cloud point method of analysis for mixtures of the utylated cresols. The cloud points can be determined rapidly ɣith ease and are duplicatable to within 0.1° C. (*2*).

For the two systems investigated, the consolute temperatures ccurred very near the 50–50 weight ratio of butylated cresol and ɔlvent (see Tables VII and XI). It is advantageous to use this articular proportion, for small errors in making up the mixtures re not serious because of the small change in temperature with omposition at this point.

The determinations were made by weighing into the Dewar est tube (the same as used for freezing point determinations) qual amounts of butylated cresol mixtures and the solvent, im-ıersing in an electrically heated bath of clear oil, and stirring ntil complete solution was effected. Then, on slow cooling nd with continued stirring, the cloud point was readily discern-ɔle. The data collected for binary mixtures of 4,6-di-*tert*-utyl-3-methylphenol, mono-*tert*-butyl-*m*-cresol, and mono-ɛrt-butyl-*p*-cresol in 2,6-di-*tert*-butyl-4-methylphenol, with di-thylene glycol as the solvent, are listed in Tables VIII, IX, and X. Determinations made with binary mixtures of 2,6-di-*tert*-butyl--methylphenol in 4,6-di-*tert*-butyl-3-methylphenol, with a 20–80 olume mixture of glycerol–ethylene glycol as the solvent, re-ulted in the data given in Table XII. Because 2,6-di-*tert*-ɔutyl-4-methylphenol is considerably less soluble in such a mixed olvent, its effect is to raise the cloud point. Diethylene glycol could not be employed as the solvent in this instance on account of its high solubility for 4,6-di-*tert*-butyl-3-methylphenol.

In the determination of these data, Eastman quality glycerol, ethylene glycol, and diethylene glycol were employed. The ethylene glycol was heated to 190° C. to drive off water before use. The solvents were characterized by the specific gravities:

Glycerol	1.260 at 80°/60° F.
Ethylene glycol	1.110 at 80°/60° F.
20% glycerol–80% ethylene glycol (by volume)	1.142 at 80°/60° F.
Diethylene glycol	1.1135 at 80°/60° F.

Small amounts of water in the solvents have considerable effect on the cloud points, and for this reason it should be emphasized that the data presented are intended to serve only as a guide. A new set of curves can be constructed readily for each batch of solvent by determination of a few key points, or the solvents might be standardized by matching definite cloud point values when mixed with selected pure tertiary butylated cresols. Table XIII exemplifies the influence of water. This pronounced effect suggests the use of cloud points, with alkylated phenols or other suitable compounds, as a method for determining accurately low percentages of water in glycols and glycerols.

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Determination of Cobalt in High-Cobalt Products

Separation from Iron by Phosphate

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A method is given in which cobalt is separated from iron in high-cobalt products by one precipitation with tribasic sodium phosphate. Since all the conditions for the reaction can be precisely controlled, the method is extremely accurate and can be used for research or referee work. Cobalt can be determined electrolytically directly following the removal of iron and aluminum by this procedure, chromium, nickel, and vanadium being the only common interfering elements. This is particularly advantageous for mining and metallurgical products, where high percentages of cobalt are frequently encountered and the resulting bulky precipitate formed with α-nitroso-β-naphthol is inconvenient to handle. The method of separating iron with zinc oxide in a volumetric flask and determining cobalt on an aliquot portion of this supernatant liquid is inaccurate, but a similar aliquoting procedure may be employed if sodium phosphate is used to remove the iron.

THE major problem confronting chemists in the analysis of cobalt is the separation of this element from iron. This is particularly important in the mining and metallurgical industry, where a high concentration of iron is usually encountered in cobalt-containing samples. Frequently, as in high-cobalt alloys, mattes, and slags, both cobalt and iron are present in large amounts.

The various methods for this separation have been reviewed and it was considered that the procedure described by North and Wells (*3*) for the precipitation of iron by sodium phosphate offered the greatest possibilities. This determination has been thoroughly investigated and various alterations in the procedure have been introduced. It is now employed in this laboratory for all research samples and for all other products where the amount of cobalt present is over 2.5 to 3.0%.

PREVIOUS WORK

In the precipitation of iron with ammonia some cobalt is always occluded in the gelatinous precipitate. Noyes and Bray (*4*) have shown that 2 mg. of cobalt are almost wholly retained in the hydroxide precipitate by 100 mg. of aluminum, iron, titanium, and zirconium, and the addition of larger quantities of cobalt leads to further occlusion. The use of potassium cyanide before precipitation of iron with ammonia produces a clean separation of the iron from cobalt, but the presence of potassium cyanide is objectionable in the subsequent determination of the cobalt.

The method developed by Fairchild (*2*), in which the trivalent metals are hydrolyzed into basic alums, also takes a considerable time and does not remove the last traces of iron from the cobalt. In the zinc oxide precipitation of iron some cobalt will always be retained in the iron precipitate unless three precipitations are carried out. It is also very difficult to be certain that the last traces of iron have been precipitated by the zinc oxide. The method for the precipitation of iron by zinc oxide does not lend itself to precise control and depends too much on judgment by the eye alone. Very accurate results can be obtained by experienced workers with the zinc oxide method, but these are largely the result of a compensation of errors in which the amount of cobalt lost is balanced by the amount of iron left in solution. It is also, of course, impossible to determine cobalt directly by electrolysis after a zinc oxide precipitation.

North and Wells (*3*) described a method in which iron is separated from cobalt by precipitation with dibasic sodium phosphate. This method permits very precise control of all the conditions during the precipitation and forms the basis of the procedure at present employed in this laboratory.

PRESENT PROCEDURE

REAGENTS. Tribasic Sodium Phosphate Solution. Dissolve 34.05 grams of reagent quality tribasic sodium phosphate dodecahydrate, $Na_3PO_4.12H_2O$, in 1 liter of water.

Cobalt Precipitating Solution. Dissolve 10 grams of α-nitroso-β-naphthol in 500 ml. of acetic acid and heat just to boiling. Add 500 ml. of water and filter.

Acetic Acid Wash Solution. Add 25 ml. of acetic acid to 1 liter of water.

Cobalt Indicator Solutions. (1) Dissolve 0.05 gram of nitroso-R-salt in 100 ml. of water. (2) Dissolve 0.5 gram of phenylthiohydantoic acid in 100 ml. of hot alcohol. Either indicator solution may be used.

SEPARATION OF COBALT

Decompose the cobalt samples in the usual way with concentrated hydrochloric and nitric acids and 1 to 1 sulfuric acid, adding a few drops of bromine or hydrofluoric acid if necessary. In the case of arsenical cobalt ores Scott and Furman (*5*) advise removing the arsenic in the decomposition by repeated treatments with hydrochloric and sulfuric acids and the addition of 1.0 gram of sodium thiosulfate.

SEPARATION FROM HYDROGEN SULFIDE GROUP OF ELEMENTS. Separate cobalt from copper, bismuth, cadmium, mercury, lead, tin, arsenic, antimony, molybdenum, etc., by gassing the acid solutions of the samples with hydrogen sulfide and filtering.

SEPARATION FROM IRON. Boil off the excess hydrogen sulfide, cool the solutions a little, and make up to approximately 350 ml. For each 0.1 gram of iron present add 25 ml. of the sodium phosphate solution plus 5 to 10 ml. in excess. For samples containing appreciable quantities of aluminum and other elements precipitated as phosphates, more sodium phosphate must be added to ensure complete precipitation. Add ammonia to the solution until it can be seen that purple cobalt phosphate is precipitating or until litmus paper turns blue. A pH of approximately 5.6 will now have been reached. Add 10 ml. of acetic acid and reduce the pH from 3.5 to 3.0. Oxidize the solution by adding 5 to 20 ml. of a 3% solution of hydrogen peroxide. Add sufficient hydrogen peroxide to make sure that all the iron is oxidized. If the amount of iron present in the sample is unknown, then add 20 ml. of the phosphate solution at first. After oxidation add further quantities of sodium phosphate solution in amounts of 5 to 10 ml. at a time until it can be seen that a small excess is present. The precipitate of ferric phosphate should come down creamy white in color.

If the precipitate of ferric phosphate is small in bulk, boil the samples for a few minutes to ensure coagulation of the precipitate, but if there is a large amount of ferric phosphate present heat the samples for only a short time; otherwise severe bumping will take place. Filter the samples through No. 31 Whatman filter papers to which a little paper pulp has been added. Wash the precipitates seven or eight times with hot acetic acid wash solution. Boil the samples to expel the excess hydrogen peroxide.

For the rapid analysis of samples with a high iron and cobalt content, filter off the ferric phosphate precipitate on a Büchner funnel or precipitate the iron in a standard liter flask. In the latter case, after precipitation make the samples up to the liter mark and mix well. When the precipitate has settled decant more than 500 ml. Filter this portion through a dry filter paper and take exactly 500 ml. for determination of the cobalt. The amount of cobalt retained in the precipitate will be very small.

DETERMINATION OF COBALT

SEPARATION BY PRECIPITATION WITH α-NITROSO-β-NAPHTHOL. Before precipitating the cobalt add 5 to 10 ml. of the sodium phosphate solution and a few drops of hydrogen peroxide to make sure that all the iron has come down. If there is any residual ferric phosphate filter the samples again. Add 4 ml. of hydrochloric acid to every 100 ml. of solution, followed by 25 ml. of the

balt precipitating solution for every 10 mg. of cobalt present. oil the samples gently for about 10 minutes and allow them to and until the precipitate has settled. Filter off the precipitate rough No. 42 Whatman paper, wash well with hot 5% hydroloric acid and finally with hot water, and ignite. Weigh the balt as Co_3O_4. If desired, the precipitate may be dissolved in ids, evaporated to dryness with sulfuric acid, and weighed as balt sulfate (*1*).

Not more than 50 mg. of cobalt can be readily precipitated by is method. Beyond that amount it appears to be difficult to sure complete precipitation by the reagent without contamination from occlusion.

Separation by Electrodeposition. Test the cobalt solutions for the presence of any residual iron by adding 5 to 10 ml. of hosphate solution and a few drops of hydrogen peroxide. Reuce to small bulk by evaporation and filter if necessary. Add 5 rams of sodium tetraborate, $Na_2B_4O_7.10H_2O$, 10 ml. of 1 to 1 ulfuric acid, and 40 to 50 ml. of ammonia.

Heat the solutions to 90° C. and electrolyze at 3 amperes and 6 olts, using a platinum gauze cathode and a rotating anode. It is etter if possible to electrolyze at 4 or 5 amperes and in no case hould the electrolysis be carried out at less than 3 amperes. etter results will always be obtained if the cobalt is plated out s rapidly as possible.

Towards the end of the electrolysis test the solutions for the resence of cobalt by either of the two indicator solutions, nitroso-R-salt or phenylthiohydantoic acid. When a drop of the solution as failed to give a red coloration with a drop of the indicator olution on a spot plate, continue the electrolysis for a further 15 o 20 minutes. Wash the cathodes in water with the current unning, then remove from the cabinet and wash in alcohol, dry, nd weigh.

The electrolytic method is preferred in this laboratory, owing o the fact that much larger amounts of cobalt can be determined. ll results quoted were obtained by this method.

RESULTS

Table I shows the results obtained on various mixtures of a balt and an iron solution. The amount of iron solution added n each case was equivalent to 0.5 gram of iron, and this was eparated from the cobalt by one precipitation with sodium hosphate.

Table I. Recovery of Cobalt from Synthetic Solutions after Separating Iron with Phosphate

Solution No.	Iron Added	Cobalt Added	Cobalt Found	Loss
	Gram	*Mg.*	*Mg.*	*Mg.*
1	0.5	40.9	40.9	Nil
2	0.5	61.3	61.1	0.2
3	0.5	81.8	81.8	Nil
4	0.5	102.3	102.3	Nil

Table II contains results on various typical Nkana samples. he results obtained by the phosphate method were checked arefully throughout for any loss of cobalt. The iron precipitates ere dissolved and tested for cobalt colorimetrically and none as found. The results are compared with those given by other orkers using the zinc oxide method. The latter results were btained only after much careful work and time had been spent n the samples.

Table II. Separation of Cobalt from Iron in Metallurgical Products with Sodium Phosphate and Zinc Oxide

Sample	Iron	Cobalt Sodium phosphate precipitation of iron	Cobalt Zinc oxide precipitation of iron
	%	%	%
Mill feed	2.38	0.14	0.14
Cobalt oxide ore	2.98	0.55	0.55
Cobalt concentrate	7.77	2.40	2.40
Cobalt reverberatory matte	13.62	3.78	3.79
Copper converter slag	40.87	4.95	4.95
Cobalt converter slag	38.10	9.44	9.45
Cobalt alloy	48.37	36.80	36.80

DISCUSSION

Ferric iron is precipitated from a solution of ferric sulfate by ribasic sodium phosphate according to the reaction:

$$2Na_3PO_4 + Fe_2(SO_4)_3 \longrightarrow 2FePO_4 + 3Na_2SO_4$$

This means that 1.0 gram of Fe^{+++} requires 6.81 grams of ribasic sodium phosphate dodecahydrate. This reaction takes place at a pH of approximately 1.6. The sodium phosphate solution is made up to contain 34.05 grams of sodium phosphate n 1 liter so that 100 ml. of this solution will precipitate 0.5 gram of Fe^{+++}. In the absence of other elements precipitated by phosphate, very little excess of the phosphate solution need be added. A necessary condition for the precipitation of iron by the phosphate in stoichiometric proportions is the complete oxidation of the iron. In any case a large excess of phosphate should be avoided; otherwise some of the cobalt is precipitated as the phosphate on the addition of ammonia before electrolysis.

The ferric phosphate precipitate is somewhat bulky and the maximum amount of precipitate which can be conveniently handled is that given by 0.5 gram of iron. For rapid analysis it is best to select a weight of sample such that not more than 0.3 gram of iron is present. For routine work large quantities of ferric phosphate may be conveniently filtered and washed on a Büchner funnel. Samples which contain a large amount of iron (60% and over) and a small amount of cobalt (2.5% and under) are analyzed in this laboratory by an ammonium thiocyanate colorimetric method shortly to be published.

It has been found by experiment that the method of adjustment of the pH for the precipitation of iron as given above gives the best results. No cobalt is retained by the iron under these conditions. It is also better to oxidize the iron after addition of the phosphate and adjustment of the pH rather than before.

The ferric phosphate precipitate should be washed with a dilute acetic acid wash solution. If water alone is used and excess phosphate is present there is a danger of precipitating some cobalt as the phosphate in the pores of the flocculent precipitate. Since ferric phosphate is insoluble in acetic acid, the iron precipitate is not redissolved by the wash solution.

INTERFERING IONS

It is beyond the scope of this paper to discuss all the ions which may have to be separated in the determination of cobalt. Ores carrying the rarer elements would have to be treated with special methods. The separation of the more common elements may be outlined here.

The separation of iron as phosphate follows prior removal of silica, together with barium and lead, by dehydration with sulfuric acid. Tungsten will also be removed in the preliminary decomposition by the usual procedures. A number of elements, such as copper, bismuth, arsenic, antimony, and molybdenum, will be eliminated by treatment with hydrogen sulfide in acid solution.

Aluminum is completely precipitated with the iron at a pH of 3.5. It must be remembered that 1 gram of aluminum requires 14.1 gram of sodium phosphate dodecahydrate for its precipitation, so that if much of this element is present a large quantity of phosphate must be added and it is also advisable to allow the samples to stand for a short time after precipitation. Zirconium and titanium will be completely precipitated with the ferric phosphate. If much titanium is present, the phosphate precipitate will be yellow in color after the addition of hydrogen peroxide. Manganese, vanadium, and chromium are not precipitated as phosphate under these conditions. Uranium is precipitated quantitatively with iron by sodium phosphate. Uranium phosphate appears to have a slightly greater occluding power towards cobalt than does ferric phosphate, and for accurate cobalt determinations in the presence of much uranium it would be necessary to dissolve and reprecipitate the uranium phosphate. Zinc may be separated from cobalt as the phosphate by carefully adjusting the pH to 4.5 to 4.8 with acetic acid by a pH meter. Nickel can-

Table III. Adsorption of Cobalt by Iron Precipitates

Precipitant for Iron	Cobalt Present	Cobalt Found	Loss
	Mg.	*Mg.*	*% of total cobalt*
Ammonia	184.0	61.2	66.74
Zinc oxide	47.2	40.8	13.56
Sodium phosphate	184.0	182.2	0.98

not be separated as the phosphate, since the pH of the precipitation, 6.3, is higher than that of cobalt, 5.3.

Few elements interfere with the determination of cobalt by α-nitroso-β-naphthol after the phosphate separation. Copper, palladium, and ferric salts are precipitated completely with the cobalt, while silver, chromium, vanadium, uranium, and bismuth are precipitated but not quantitatively. The more common elements which are likely to interfere are either removed with the hydrogen sulfide group or as phosphate with the iron, except chromium and vanadium. These two elements may be eliminated by a sodium hydroxide separation. Zinc, manganese, and nickel do not interfere. If there are large quantities of other elements present, however, the voluminous precipitate of cobalt is apt to adsorb other ions, and it is then advisable to carry out this precipitation twice.

The ions of some metals will interfere with the electrolysis of cobalt either by depositing with it or by precipitating as hydroxides or phosphates which retain cobalt. Unless zinc has previously been completely separated it will form complex cations in excess ammonia and be deposited with the cobalt. Nickel will also be deposited with the cobalt and may be determined by dissolving the cathode deposit in nitric acid and separating with dimethylglyoxime in the usual manner. If a large excess of phosphate has been used in precipitating iron, calcium, strontium, and magnesium will be precipitated in the ammoniacal electrolytic solution and may occlude some cobalt. If a large quantity of calcium is present it may be precipitated as the oxalate after the phosphate separation. If much magnesium is present an excess of phosphate must be rigorously avoided. Chromium will be precipitated as the hydroxide in the ammoniacal electrolytic solution. Since this precipitate may occlude some cobalt, the chromium should be previously separated with sodium hydroxide and sodium peroxide. If large quantities of manganese are present they may be removed by initial treatment with nitric acid and potassium chlorate, or the separation of cobalt and manganese phosphates can be carried out in the presence of ammonium citrate (*1*). When small quantities of manganese are fully oxidized and the electrolysis is not unduly prolonged after deposition of all the cobalt, manganese will not deposit on the cathode, provided also that the concentration of ammonia in the electrolytic solution is kept high. Vanadium forms a complex with cobalt in the ammoniacal electrolytic solution and it is then impossible to deposit any of the cobalt on the cathode. Vanadium should be previously separated with sodium hydroxide and sodium peroxide.

COMPARATIVE RETENTIVE POWERS OF IRON PRECIPITATES

Iron was separated from cobalt in various samples by the method of precipitation in a liter flask and decantation. The precipitants used were ammonia, zinc oxide, and sodium phosphate solution. The amount of iron present varied from 0.4 to 0.5 gram. The results are given in Table III.

Many analytical texts recommend the separation of iron from cobalt by the addition of zinc oxide to a volumetric flask containing the solution of the sample, followed by the withdrawal of an aliquot portion of the supernatant liquid for the determination of cobalt. Table III indicates that this practice may be unsatisfactory even for routine work. On the other hand, sodium phosphate can be used for all routine work in this manner, since the adsorptive power of ferric phosphate for cobalt is shown in Table III to be very low.

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Colorimetric Determination of Cobalt with Ammonium Thiocyanate

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A rapid and accurate colorimetric method is given whereby cobalt may be determined in the presence of other common ions. This method can be extremely valuable in the laboratories of mining and metallurgical industries where routine determinations of cobalt are carried out. The useful range for this procedure lies within the limits 0.01 to 4.0% cobalt.

THE alkali thiocyanates have been used by many workers to detect the presence of small quantities of cobalt. Potassium and ammonium thiocyanates form complexes with cobalt which are represented by the formulas $K_2Co(SCN)_4$ and $(NH_4)_2Co(SCN)_4$. These complexes may be extracted from aqueous solutions by organic solvents. A rapid and accurate method for the determination of cobalt in ores and concentrates has been developed in the Nkana laboratory by comparing the blue color of these extracts of the cobalt complex by either visual or photoelectric means. The method has been investigated in great detail and applied successfully to the determination of cobalt in various mining and metallurgical samples in Northern Rhodesia for the past three years.

The alkali thiocyanate complexes of cobalt were first described by Skey (*6*), Morrell (*4*), and Vogel (*9*) in the years 1868 to 1879. Since that time a number of papers have appeared on this subject, but the most notable contributions have been presented by Treadwell (*8*), Powell (*5*), Feigl and Stern (*1*), Tomula (*7*), and Gorski (*2*).

Treadwell treated a solution of a cobalt salt with concentrated ammonium thiocyanate and extracted the complex with amyl alcohol or with a mixture of one part of amyl alcohol to one part of ether. A pure extract of the complex shows a characteristic absorption spectrum. If ferric iron is present in the cobalt solution, ferric thiocyanate is formed which colors the alcohol extract red. The iron may be removed by treatment with sodium hydroxide. Treadwell quotes Vogt's analysis of the ammonium thiocyanate complex which gives Co = 18.01%, S = 39.15%, and NH_4 = 10.42%. This is calculated to the formula $(NH_4)_2Co(SCN)_4$. This complex is easily decomposed even by damp air to give cobalt and ammonium thiocyanates.

Powell considered the minimum concentration of ammonium thiocyanate necessary for the formation of the cobalt complex was 25% and preferred to use 30%. Varying results were obtained according to the acidity or alkalinity of the cobalt solution. Sodium pyrophosphate may be used to suppress the color due to iron.

Feigl preferred acetone as a solution medium for the complex in drop reactions for the detection of cobalt. The color of the complex is stronger if saturated solutions of ammonium thiocyanate are used. The fixation of iron may be brought about by the addition of phosphate, but all the methods for the suppressio

the color due to iron are effective only if the latter is present in uch less amount than the cobalt.

Tomula carried out experiments to determine the maximum ncentration of thiocyanate which was necessary to produce the eatest intensity of color of the complex. For a cobalt concenation of 1.192×10^{-4} mole of cobalt chloride per liter a 5% ncentration of thiocyanate was sufficient. Acetone may be prerred as a solution medium for the complex. Nickel salts form a een complex with the thiocyanate which is not soluble in acene, but the presence of this green complex necessitates the use yellow filters in the colorimeter. Tomula quotes the work of itz as showing that the capacity of a solvent for preventing demposition of a complex varies inversely as its dielectric conant.

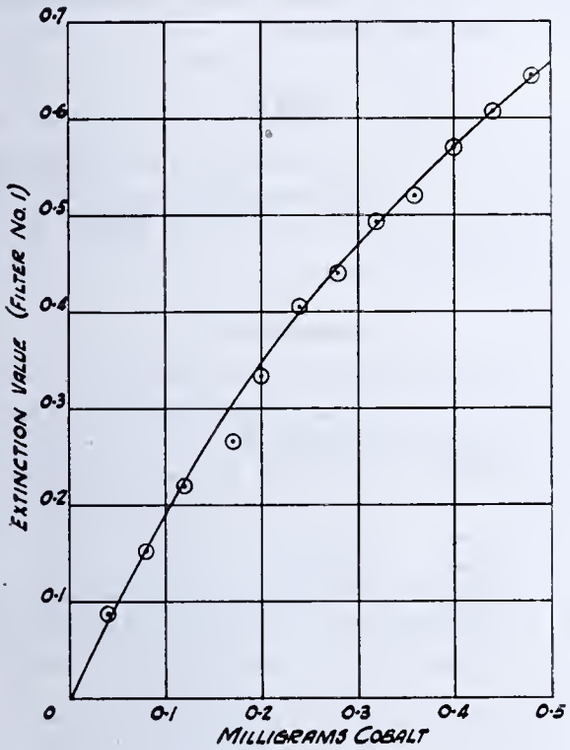

Figure 1. Calibration Curve for Ammonium Cobaltothiocyanate

In the method given by Gorski for the detection of small nounts of cobalt 15 ml. of ethyl alcohol and 5 ml. of a 5 *N* soluon of potassium or ammonium thiocyanate are added to 5 ml. the test solution. The concentration of thiocyanate in the ixture is now 1 *N*. By this method 0.003 mg. of cobalt may be etermined.

REAGENTS

Ammonium Thiocyanate Solution. Dissolve 600 grams of nmonium thiocyanate, NH_4CNS, in 1 liter of water.

Sodium Phosphate Solution. Dissolve 83.3 grams of tribasic dium phosphate dodecahydrate, $Na_3PO_4.12H_2O$, in 1 liter of ater.

Sodium Thiosulfate Solution. Dissolve 200 grams of sodium iosulfate, $Na_2S_2O_3.5H_2O$, in 1 liter of water.

Combined Sodium Phosphate and Thiosulfate Solution. To crease the speed of the analysis the sodium phosphate and thiolfate solutions may be combined in one. It is not advisable to clude the ammonium thiocyanate in this also. Dissolve 125 ams of $Na_2S_2O_3.5H_2O$ and 31.25 grams of $Na_3PO_4.12H_2O$ in 1 er of water.

Ammonium Acetate Solution. Dissolve 700 grams of ammoum acetate, $NH_4C_2H_3O_2$, in 1 liter of water.

Tartaric Acid Solution. Dissolve 50 grams of tartaric acid, H_6O_6, in 100 ml. of water.

Amyl Alcohol–Ether Mixture. Mix 3 parts by volume of amyl cohol with 1 part of ethyl ether.

METHODS OF COMPARISON

Visual. The color of the cobalt thiocyanate complex fades ter a short time and therefore a permanent set of standards nnot be made up from organic solutions of the complex. Solutions of copper sulfate may be used as permanent standards, since the blue color of the copper sulfate solutions matches almost identically the blue of the cobalt complex. Copper sulfate solutions are made up to match varying amounts of cobalt and are kept in sealed test tubes. The color of these will last for a considerable period, but the standards should be checked against known amounts of cobalt from time to time.

A solution of copper sulfate in water containing 8 grams of cupric sulfate pentahydrate per liter will match an extract containing 0.02 mg. of cobalt per 10 ml. By progressively increasing the strength of the standard solutions by 8 grams of cupric sulfate pentahydrate, a range of standards is obtained which will match extracts of the cobalt complex at intervals of 0.02 mg. of cobalt. It has not been found practicable to take this set of standards beyond 0.40 mg. of cobalt. This means that the final standard will contain 160 grams of cupric sulfate pentahydrate per liter.

In Table I are given the weights of samples taken and the dilutions according to the percentage of cobalt present in the sample.

The number of milligrams of cobalt present in an extract is ascertained by matching the color with that of the standard solutions. To obtain the percentage of cobalt present in the sample this number of milligrams is taken multiplied by one of the factors given in the final column of Table I according to the weight of sample taken and the dilution. For example, let us assume that a 0.5-gram sample is taken, and its solution is diluted to 50 ml. From this solution 5 ml. are taken to give the cobalt complex. The extract of this complex matches a standard corresponding to 0.24 mg. of cobalt. By multiplying 0.24 mg. by 2 a percentage of 0.48 for cobalt is obtained. Since 0.24 mg. of cobalt were present in the aliquot the percentage of cobalt in the sample is seen to be

$$0.00024 \times 10 \times \frac{100}{0.5} = 0.48\%.$$

Photoelectric. A calibration curve for the color intensities of the extracts of the cobalt complex may be drawn up by means of an absorptiometer.

For this work a Spekker photoelectric absorptiometer was used with 1-cm. glass cells and Spekker red filter No. 1. The absorption of light by the complex was in each case compared with the absorption of a blank mixture of amyl alcohol and ether extracted from the reagents. In the construction of the curve 10-ml. extracts of the cobalt complex ranging from 0.02 to 0.50 mg. of cobalt were used. In this case the increment of cobalt content was again 0.02 mg. Only the intermediate points of the calibration curve are shown in Figure 1.

Table I. Weights and Dilutions of Samples According to Percentage of Cobalt Present

Cobalt in Sample	Sample Taken for Analysis	Dilution	Aliquot Taken for Analysis	Factor for % Cobalt
%	*Grams*	*Ml.*	*Ml.*	
0.01 to 0.20	2.0	50	5	0.5
0.16 to 0.80	0.5	50	5	2
0.80 to 3.20	0.5	200	5	8

PROCEDURE

Decompose the cobalt samples with 10 ml. of nitric acid and 20 ml. of hydrochloric acid, adding a few drops of bromine or hydrofluoric acid if necessary. Decompose samples having a high iron content with a solution of nitric acid and potassium chlorate. Take the samples down to dryness but do not bake. Traces of nitric acid have no effect on the formation of the thiocyanate complex.

Take up the samples in approximately 25 ml. of water, and add exactly 1 ml. of hydrochloric acid for every 50 ml. of subsequent dilution of the sample. To effect solution boil the samples gently for a few minutes. Cool the solutions and wash out into appropriate measuring cylinders or calibrated flasks. The amount of dilution of the samples depends on the amount of cobalt present and can be read off from Table I.

After dilution the pH of the sample solutions is 1.0 to 0.9. This is one of the most important steps in the analysis. In samples where the percentage of cobalt is entirely unknown and the first dilution attempted has proved insufficient, it is of no use to try further dilution unless the pH is adjusted by the careful

addition of more acid with the help of a pH meter. It is better otherwise to weigh out another and smaller amount of sample.

Cobalt Samples with Less Than 40% Iron. Measure out 5 ml. of sodium thiosulfate solution, 3 ml. of sodium phosphate solution (or 8 ml. of the combined sodium thiosulfate and phosphate solution), and 10 ml. of ammonium thiocyanate solution. Add with vigorous agitation 5 ml. of the solution of the sample. The pH is now 3.5 to 4.0 and the concentration of ammonium thiocyanate is 26%.

Table II. Gravimetric and Colorimetric Determinations of Cobalt in Metallurgical Products

	Per Cent Cobalt		
	Gravimetric	Colorimetric	Difference
Mill tails	0.06	0.06	0.00
Mill feed	0.14	0.14	0.00
Cobalt oxide ore	0.55	0.56	+0.01
Primary cobalt concentrate	0.70	0.68	−0.02
Secondary cobalt concentrate	3.04	3.04	0.00
Cobalt concentrate	2.40	2.40	0.00
Copper concentrate	0.64	0.64	0.00
Cobalt gravity concentrate	10.15	10.00	−0.15
Cobalt gravity tails	0.21	0.20	−0.01
Copper reverberatory slag	1.47	1.44	−0.03
Cobalt reverberatory slag	0.63	0.64	+0.01
Cobalt reverberatory matte	3.79	3.84	+0.05
Electric furnace slag	2.21	2.24	+0.03
U. S. Bureau of Standards No. 115	0.08	0.08	0.00

Add 10 ml. of the amyl alcohol–ether mixture and shake the whole thoroughly again. Transfer to a separatory funnel, run off the lower aqueous layer, and discard. Transfer the solution of the cobalt complex to a test tube or a 1-cm. absorptiometer cell. For visual comparison match the intensity of color of the test solution with the standard copper sulfate solutions. The comparison may be carried out in a LaMotte comparator for hydrogen-ion determinations with a source of artificial light, or the tubes may simply be held against a white background out of direct sunlight. It will be found possible to take a reading halfway between any two standards if the color of the test solutions lies between them.

In the photoelectric comparison, absorption of the test solution is compared with an amyl alcohol–ether blank. The amount of cobalt present in the test solution is then read off from the calibration curve.

By either means of comparison the amount in milligrams of cobalt present in the extract is obtained and the percentage of cobalt in the sample is given by multiplying by the appropriate factor shown in the last column of Table I.

Cobalt Samples with More Than 40% Iron. Carry out the analysis of these samples in exactly the same way as for samples with less iron, but in this case add 2 ml. of ammonium acetate solution and 3 drops of tartaric acid solution. The pH is still 3.5 to 4.0 and the concentration of ammonium thiocyanate is 24%.

INTERFERING IONS

Copper does not interfere with the production of the blue color of the cobalt complex even when it is present in amount equaling 60% of the sample.

Iron, if present in amount greater than 40% of the sample, will interfere unless ammonium acetate and tartaric acid are used.

Chromium, manganese, nickel, zinc, titanium, molybdenum, and uranium do not give colored complexes which are soluble in amyl alcohol and ether. Other common elements such as silica, aluminum, calcium, magnesium, phosphorus, bismuth, arsenic, lead, and the alkalies are without effect.

Vanadium under these conditions also forms a blue complex which is extracted by the amyl alcohol–ether solution. If, however, ammonium acetate and tartaric acid are added to the reagents this blue complex is not formed, and vanadium will not interfere with the determination of cobalt.

SENSITIVITY

The smallest amount of cobalt which can be conveniently determined by either visual or photoelectric comparison is 0.02 mg. It can be seen from the calibration graph obtained from the absorptiometer readings that the relationship between the color intensity of the cobalt complex and the cobalt content of the extracts is not linear but follows a curve of wide radius. The last three standards employed in the visual comparison are slightly more intense in color than corresponding extracts of cobalt, so that accurate determinations cannot be carried beyond 0.40 mg. of cobalt at the most. By use of an absorptiometer accurate determinations can be obtained up to 0.50 mg. of cobalt.

Using the range 0.02 to 0.50 mg. of cobalt, accurate determinations have been carried out on Nkana samples between 0.01 and 4.00% cobalt. By taking smaller weights of sample, it has been possible to estimate cobalt up to 10% with a sufficient degree of accuracy for rapid works practice.

RESULTS

A number of results of the colorimetric method, obtained by both visual and photoelectric comparisons, are given in Table II, compared with the percentages of cobalt obtained by careful gravimetric analyses.

DISCUSSION

A pH of 1.0 to 0.9 for the solution of the sample was found by experiment to be the optimum for the formation of the cobalt complex. If the pH is higher than this the color is not so strongly developed, while if it is lower it is impossible to suppress with small amounts of phosphate the color due to iron. A final pH of 3.5 to 4.0 in the solution of reagents and sample is well below the precipitation pH of cobalt phosphate, which is about 5.3.

Sodium thiosulfate has been found to be an efficient reducing agent for the iron. Other reagents such as stannous chloride and hydrogen sulfide gas have been tried out but were not found so satisfactory.

A concentration of 24 to 26% ammonium thiocyanate is sufficient to produce the maximum color intensity for amounts of cobalt ranging from 0.02 to 0.40 mg. For quantities of cobalt up to approximately 0.14 mg. a concentration of 17% ammonium thiocyanate is sufficient, but beyond this 26% concentration must be used. It is not so convenient in routine analyses to alter the concentration of a reagent according to the samples and therefore a standard concentration of 24 to 26% ammonium thiocyanate has been given in the procedure. Increasing the amount of thiocyanate beyond 26% did not further intensify the color.

The dielectric constant of amyl alcohol at 0° C. and infinite wave length is 17.4 (*3*). The dielectric constant of ethyl ether is 4.68. A mixture of these two organic liquids will then have a lower dielectric constant and greater capacity for preventing decomposition of the complex than methyl alcohol, ethyl alcohol, or acetone which have dielectric constants of 35.0, 28.4, and 26.6, respectively. A mixture of 3 parts by volume of amyl alcohol to 1 part of ether has been found to give a maximum intensity of color.

The use of ammonium acetate and tartaric acid solution makes it possible to determine cobalt on samples high in iron and vanadium. By this method small amounts of cobalt may be determined in steels and in the iron precipitates in a gravimetric analysis of cobalt.

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Most Economic Sampling for Chemical Analysis

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IN AN expository article (*3*), Mandel has outlined the application of modern statistical methods to chemical experiments. It is the purpose of this paper to acquaint the chemist with a recently developed technique of analysis of data, known as "sequential analysis", that has important application to chemical analyses. The new method is designed to enable the experimenter to reach a decision with the minimum number of observations, and is based upon (*6*) and (*7*). The interested reader will find it profitable to obtain these papers, since their application is more general than the subject matter of this paper; a more extended memorandum on the present subject has also been issued (*2*).

The problem of interest here is the following:

The experimenter, by his qualitative and elementary analysis and molecular weight determinations, together with certain theoretical considerations, has decided that the compound is one of two or more possibilities; how many determinations shall he make in order to obtain a certain degree of confidence in his analysis?

This problem is of wide application, as can be seen from the following notes of H. Gisser, Frankford Arsenal Ordnance Laboratory, who suggested the need for an exact mathematical procedure in these matters:

In the study of Diels' hydrocarbon (from selenium dehydrogenation of cholesterol) the first suggested formula was $C_{18}H_{16}$. From available analyses, Ruzicka (*5*) suggested it might be $C_{17}H_{14}$. Later work proved $C_{18}H_{16}$ correct. Elucidation of the structure of retene ($C_{18}H_{18}$) was delayed because of poor analytical results. Fieser reports that although derivatives and degradation products of retene had been prepared in pure condition, the relationship between them remained obscure until the analytical investigations of Bamberger and Hooker (*1*) cleared up the empirical formulas. The formulas previously assigned required revision. Concerning the structure of morphine, the following estimates are found in the literature: Liebig (1831) $C_{34}H_{36}O_6N_2$; Regnault (1838) $C_{35}H_{40}O_5N_2$; Laurent (1847) $C_{34}H_{38}O_6N_2$, the last corresponding to the present accepted formula $C_{17}H_{19}O_3N$. On the structure of a papaverine Merck gave (1848) $C_{20}H_{21}O_4N$; Hesse gave (1870) $C_{21}H_{21}O_4N$, and his results were confirmed by other laboratories; Merck's formula was later shown to be correct.

PRELIMINARY REMARKS

In the application of statistical methods to the problem of determining the formulas of organic compounds, the following is presupposed:

1. The formula is one of two possibilities. From these two possibilities, the chemist can calculate the expected percentage for each element (or for some derivative compound).
2. The variance has been calculated for each set of readings. As in (*1*), the estimate of the variance is given by the formula:

$$S^2 = \frac{\sum_{\alpha=1}^{N} (x_\alpha - \bar{x})^2}{N-1}$$

where Σ stands for a summation, x_α for any of the series of observations, N for the total number of observations, and $\bar{x}$ for the arithmetic mean. It is further presupposed that there has been gathered a considerable amount of data, and that these data plus the underlying chemical theory are sufficient to establish with some degree of confidence the following:

3. That the observations obey a specific normal, or Gaussian law of error.
4. That the estimate of the variance is "very nearly" exact.

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No estimate can be exact at any finite stage of scientific progress; it is therefore a matter of some difficulty to specify the degree of precision that must be obtained. In general, the accuracy one should obtain before applying the procedures of this paper depends upon the fineness of the distinction that the experimenter wishes to determine. As a practical suggestion, the variances should be computed on the basis of 200 or more observations, all made essentially under the same conditions—i.e., all presupposed to have the same error. It is to be noted that the variances obtained from different experiments may be averaged, provided the errors of observation are assumed to be the same. There are to be found in the statistical literature certain tests for the homogeneity of a set of variances, to be applied when some doubt exists concerning the advisability of averaging.

These remarks might lead the chemist to disregard all exact procedures, on the grounds that he can never reach the kind of precision the mathematical statistician demands. This is an all too common mistake in the experimenter's attitude towards statistical procedures, a mistake that is sometimes encouraged by the statistician himself. One would be foolish indeed to expect that all observations are governed by exactly the same law of error, and that the errors of determination are really fixed for all observers. The conditions under which the statistician's procedures are outlined are ideal or formal, and the conditions under which the chemist operates are practical or nonformal. If the experimenter waits for the ideal to show up before using statistical procedures, he will have to wait forever. Rather, the experimenter must use his own judgment, based on his and others' extensive experience, together with the cooperation of the statistician, to decide whether or not a given procedure is feasible. To fail to apply the best procedures available, because their conditions cannot be met exactly, is as absurd as failing to use a certain machine because its cogs do not operate exactly as designed. In the sequel, we will symbolize known variance by σ^2.

PROCEDURES

In the procedure, we shall consider two cases, one in which all the information is to be obtained from one type of analysis, the other where at least two different types of analysis are made.

CASE 1. Suppose then, that the experimenter decides that the compound is one of two possibilities, X and Y, and that he selects for study, say, one of the elements A (or else the molecular weight). The two possibilities will determine values for this element A—that is, granting X as the true formula, one can calculate the expected percentage of A, say α_1, and granting Y as the true formula, one can calculate the expected percentage of A, say α_2. Let σ_α^2 be the variance of percentage determinations of A. In general, the element A which should be selected for study is that one which makes the value $(\alpha_2 - \alpha_1)^2/\sigma_\alpha^2$ the largest, for the larger this value, the fewer the expected number of determinations necessary to reach a decision. However, if A is difficult to determine, it may be desirable to sacrifice economy of sample size for economy of time and expenditure, and some other element will be selected.

The procedure for this case follows:

Let $\overset{n}{\Sigma}\alpha$ represent the sum of the α determinations up to and including the nth determination. Thus if the percentages for hydrogen were 5.02, 5.10, and 5.06 for the first three observations, we would have $\overset{1}{\Sigma}H = 5.02$, $\overset{2}{\Sigma}H = 10.12$, $\overset{3}{\Sigma}H = 15.18$. Calculate

$$h = \frac{4.595}{\alpha_2 - \alpha_1}\sigma_\alpha^2$$

and

$$s = \frac{\alpha_2 + \alpha_1}{2}$$

where $\alpha_2 > \alpha_1$.

Accept the hypothesis that the true percentage, α, is α_1 as soon as

$$\sum^{n} \alpha \leq sn - h \ (n = \text{number of samples})$$

Accept the hypothesis that the true percentage, α, is α_2 as soon as

$$\sum^{n} \alpha \geq sn + h$$

If $\sum^{n} \alpha$ lies between $sn - h$ and $sn + h$, take an additional observation and repeat the procedure until enough readings have been taken.

CASE 2. Consider now the case where the experimenter decides to choose more than one element to investigate. Suppose that there are two possibilities open, and independent tests have been run on A and B; possibility X requires a value α_1 for A and β_1 for B; possibility Y requires a value α_2 for A, and β_2 for B. Let n be the number of determinations on α and m the number on β. As before, let σ_α^2 be the variance for determinations of α, σ_β^2 be the variance for β determinations. The function

$$\left[\frac{\alpha_2 - \alpha_1}{\sigma_\alpha^2}\right]\sum^{n}\alpha + \left[\frac{\beta_2 - \beta_1}{\sigma_\beta^2}\right]\sum^{m}\beta - n\left[\frac{\alpha_2^2 - \alpha_1^2}{2\sigma_\alpha^2}\right] - m\left[\frac{\beta_2^2 - \beta_1^2}{2\sigma_\beta^2}\right]$$

is "critical" here; if it is less than or equal to -4.595, then accept possibility X, and if it is greater than or equal to $+4.595$, accept possibility Y. If it lies between -4.595 and $+4.595$, make another determination on α or β or both. The extension of this function to cases where more than two elements are independently investigated is obvious. Also note that the molecular weight can be included. It is important to emphasize that the values of α and β in the procedure must have been determined independently—for example, β should not be determined by subtraction of α from 100.

If the above procedures are followed, it can be shown that the expected number of determinations necessary to reach a decision is less than that of any other test procedure involving the same risks of error. It has been assumed throughout that a confidence of 0.99 is sufficient for these determinations—i.e., an error would be committed no more frequently than one time in a hundred in the long run; if greater or less confidence is desired, the procedure is only modified by replacing the number 4.595 throughout by another figure; the following table may be convenient in this connection:

Confidence Level	Value to Replace 4.595
0.999	6.907
0.98	3.892
0.95	2.944
0.90	2.197
0.80	1.386

The significance of this table is explained as follows: Every experiment is designed to enable us to choose some hypothesis about the natural world, and to reject alternative hypotheses. In the case at hand, the hypotheses are concerned with the correct chemical formula of a given compound. Now no experiment is "ideal" in the sense that one hypothesis can be selected without any possibility of error. Rather, any method we may use for choosing one hypothesis over the others on the basis of the data at hand will in the long run lead to mistaken choices. The advantage of statistical procedures in general lies in the fact that they enable us to estimate the frequency of wrong decisions for a given method, and that they propose methods that in some sense "minimize" this frequency of wrong choices. If the above procedure is used, and the value of 4.595 is employed, then the chance of accepting possibility X when possibility Y is actually true is 0.01—i.e., we go wrong in this way, in the long run, one time in a hundred. Similarly, the chance of accepting possibility Y when possibility X is actually true is 0.01. If 6.907 is used, the chances in each case are 0.001; if 3.892 is used, the chances are 0.02, etc. Now these estimates of the chance of a wrong decision depend upon the presuppositions we make, particularly the presupposition of normality of distribution, and the accuracy of the variance. This is the manner in which these initial assumptions are important; as mentioned before, we cannot ever be sure that the assumptions are true, and hence the estimates of the chances of making a mistake are only approximations, the best approximations that can be made for a given state of our knowledge.

EXAMPLE

The following is taken from (*4*). Two possibilities were open here:

X $C_{19}H_{12}O_6Br_4S$ (Carbinol sulfonic acid)

Y $C_{19}H_{10}O_5Br_4S$ (Lactoid)

Bromine and sulfur were selected for analysis. Hence this example falls under Case 2. The two hypotheses were

$$X \ Br_2 = 46.47; \ S_2 = 4.66$$

$$Y \ Br_1 = 47.72; \ S_1 = 4.79$$

Two determinations were made independently for each element with the following results:

Bromine 47.75, 47.70
Sulfur 4.76, 4.88

Were enough determinations made? The errors of estimate are not given, but to complete the example we suppose that the standard deviations are about 0.500%, in each case. This gives a variance of $(0.5)^2 = 0.25$. (Note. This value of the error of a single determination is supposed only for the sake of illustration; actually, the errors are probably smaller.)

We treat the problem sequentially, by considering the results of the first determinations, Br = 47.75, S = 4.76. We have

$$\sum^{1}\text{Br} = 47.75$$

$$\sum^{1}\text{S} = 4.76$$

$$n = m = 1$$

$$Br_2 - Br_1 = 1.25$$

$$(Br_2 - Br_1)/\sigma_{Br}^2 = (1.25)/0.25 = 5.0000$$

$$S_2 - S_1 = 0.13$$

$$(S_2 - S_1)/\sigma_S^2 = (0.13)/0.25 = 0.5200$$

$$Br_2^2 - Br_1^2 = 117.7375$$

$$(Br_2^2 - Br_1^2)/2\sigma_{Br}^2 = 117.7375/0.5 = 235.4750$$

$$(S_2^2 - S_1^2)/2\sigma_S^2 = 1.2285 = 2.4570$$

We have, then, as the critical function

$$5\sum^{n}\text{Br} + 0.52\sum^{m}\text{S} - 235.4750\,n - 2.4570\,m$$

(Note that in the subsequent determinations, only $\Sigma\alpha$ and $\Sigma\beta$ have to be calculated, since the values 5, 0.52, 235.475, and 2.4570 remain the same throughout.) For $n = m = 1$, the critical function is

$$5(47.75) + 0.52(4.76) - 235.4750 - 2.4570 = 3.2932$$

The value 3.2548 lies between -4.595 and $+4.595$, and hence the first pair of determinations was not sufficient, and additional determinations must be taken. For $n = m = 2$, we have

$$\sum^{2}\text{Br} = 95.45$$

$$\sum^{2}\text{S} = 9.64$$

$$n = m = 2$$

Hence the critical function is

$$5(95.45) + 0.52(9.64) - 2(235.4750) - 2(2.4570) = 6.3988$$

The value 6.3988 is greater than 4.595, and hence possibility Y (lactoid) should be accepted.

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Drill Sampling Device for Fish Livers

Precision and Accuracy

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Because of the unhomogeneous nature of fish livers, errors in vitamin A assays due to sampling may be large unless an adequate number of cores are taken. In developmental work on a drill sampling device in which over 2000 cans of soupfin shark and grayfish livers were tested, 100 cores per ordinary commercial lot were sufficient to give vitamin A assays that were reproducible within 5%. An investigation of the accuracy of the sampling showed that any bias, if present, was of small magnitude. Essentially the same analytical results were obtained whether the livers were fresh or frozen when sampled. The sampling was rapid. A hundred cores could be taken in 15 minutes or less, and the rate at which the livers were cored could be varied within wide limits without appreciably affecting precision.

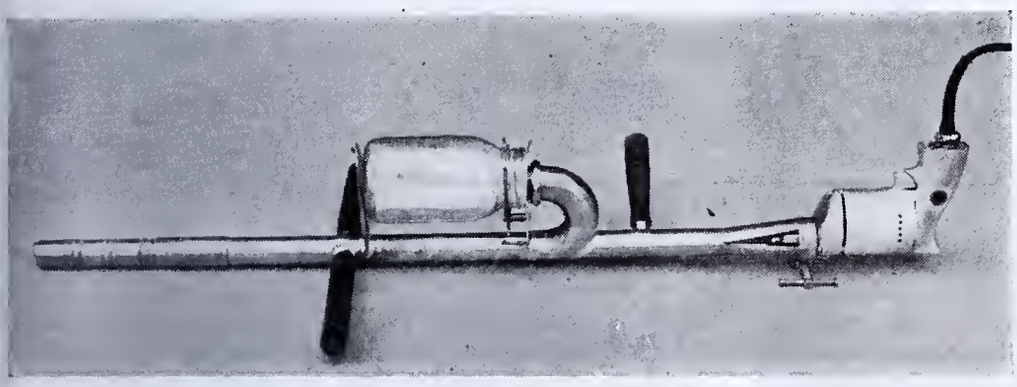

Figure 1. Motor-Driven Core Sampler

A SURPRISING feature of the fish liver industry has been the practice of "blind buying". Livers are bought from the fishermen without the benefit of a test for vitamin content. The buyer may examine the livers visually (dark-colored livers usually contain more vitamin A than light-colored livers), but since the variation in the appearance of the livers is not commensurate with the variation in their potency, visual inspection will detect only differences of large magnitude. This practice has persisted even though livers for some of the species, such as male soupfin shark, may vary in value per pound from as low as 25 cents to as high as $12 or $13. Obviously, liver trading on the basis of "blind buying" is extremely hazardous.

A primary difficulty interfering with sales on a potency basis has been the problem of sampling. While hard-frozen livers can be sampled with conventional core samplers, a method has been needed whereby the "fresh" livers could be sampled just as they came from the fishing vessel. (If the livers are not frozen, salted, or otherwise preserved, they are called "fresh" by the trade, even though they may actually be stale.) The fishermen are ordinarily at sea for several days, and trips of 3 or 4 weeks' duration are not uncommon. When fishing conditions are favorable, the fishermen are anxious to put out to sea again; understandably, they are not willing to delay long in settling their business. For this reason, the freezing of livers in preparation for sampling, while practiced extensively in sales between liver buyers and processors, has never been popular in primary sales, since a delay of several days often occurs while the livers are being frozen.

To meet the need for a rapid method of sampling "fresh" livers, this laboratory has developed a motor-driven core sampler (Figure 1) consisting of a tube within which a tightly fitting, rotating auger elevates the liver material into a sample bottle (*1*, *3*). Figure 2 shows shark livers that had just been landed in the process of being sampled by a commercial processor with a sampler fabricated in his own shop. In the course of the development of the sampler over 2000 cans of soupfin shark (*Galeorhinus zyopterus*) and grayfish (*Squalus sucklii*) livers were tested. The present paper reports the results of this work.

A previous study (*2*) of a somewhat theoretical nature showed that taking a large number of cores is required for proper sampling and that, in general, it is necessary to take at least 100 cores from an ordinary commercial lot if the precision is to be within +5%. However, conditions vary greatly. Not only are there differences of texture in the livers of various species such as soupfin shark, grayfish, ling cod, halibut, and tuna, but the texture also varies within even a single species. Some livers will be fresh and firm; others will be stale, soft, and perhaps partially decomposed. In addition, there may be other differences in the livers, attributable to seasonal or geographic causes, so that the number of variables involved in sampling is too large to allow for a rigorous determination of precision under all circumstances. Consequently, in order to test the conclusions of the earlier theoretical studies, it has been necessary to adopt an empirical procedure in the study of liver sampling and to limit these experiments to livers landed in the Pacific Northwest.

Figure 2. Motor-Driven Core Sampler in Use

Some shark livers are exposed to show nature of material

Table I. Reproducibility of Samples of Fresh Grayfish (Dogfish) Livers

(One core taken per can)

Total Cans in Lot	Sample No.	Oil Content, %	Deviation of Oil Content from Average, Relative %	Oil Potency, U.S.P. Units Vitamin A per Gram	Deviation of Oil Potency from Average, Relative %	Liver Potency, Millions of Units Vitamin A per Lb.	Deviation of Liver Potency from Average, Relative %
16	1	72.5	0.96	13,600	0.74	4.48	0.00
	2	73.1	0.14	12,600	6.67	4.18	6.70
	3	73.9	0.96	13,500	0.00	4.53	1.12
	4	73.3	0.14	14,800	9.63	4.92	9.82
	5	73.0	0.28	12,900	4.44	4.28	4.46
	Av.	73.2	0.50	13,500	4.30	4.48	4.42
27	1	74.6	1.91	17,400	6.97	5.90	5.15
	2	73.3	0.14	18,600	0.54	6.20	0.32
	3	73.1	0.14	19,100	2.14	6.34	1.93
	4	71.6	2.19	19,400	3.75	6.30	1.29
	5	73.5	0.41	19,000	1.61	6.34	1.93
	Av.	73.2	0.96	18,700	3.00	6.22	2.12
40	1	66.6	1.04	7,710	0.39	2.33	1.70
	2	68.0	1.04	7,620	1.55	2.35	0.84
	3	67.6	0.45	7,620	1.55	2.34	1.27
	4	66.9	0.60	8,110	4.78	2.46	3.80
	5	67.5	0.30	7,660	1.03	2.35	0.84
	Av.	67.3	0.69	7,740	1.86	2.37	1.69
56	1	69.4	0.73	5,220	2.97	1.65	1.78
	2	69.6	1.02	5,420	0.74	1.71	1.78
	3	67.9	1.45	5,640	4.83	1.73	2.98
	4	68.7	0.29	5,220	2.97	1.62	3.57
	Av.	68.9	0.87	5,380	2.88	1.68	2.53
135	1	70.9	0.28	14,200	0.70	4.57	0.87
	2	70.6	0.70	13,900	2.80	4.46	3.25
	3	71.8	0.98	14,300	0.00	4.66	1.08
	4	70.9	0.28	14,800	3.50	4.76	3.25
	Av.	71.1	0.56	14,300	1.75	4.61	2.11

In forming a sample for purposes of study, one core was taken from each can in the lot, and the cores were then composited. Additional samples from the lot were formed in the same manner. Where the number of cans in the lot was small, the assays tended to be somewhat divergent. However, even in one case (Table I) where the lot contained only 16 cans, giving only 16 cores per sample, the greatest deviation from the average of 5 samples from this lot did not exceed 10%. In general, as can be seen from Tables I and II, when lots containing a fairly large number of cans were sampled, assays of both "fresh" grayfish and soupfin shark livers were reproducible to within 5%.

Livers and viscera are received in so many different conditions that it is beyond the scope of this laboratory to test them under all circumstances. However, a few experiments have been performed with livers from halibut, ling cod, rock cod, and sole, as well as with halibut viscera. These lots were all soft-frozen. While it was not possible to make an extensive test of precision, the samples appeared to be representative, and no difficulties were encountered. In Table III are reported the results of sampling 35 cans of soft-frozen tuna (*Germo alalunga*) livers. These data show that the sampler, at least with frozen livers, will work well with species other than the grayfish or soupfin shark.

A question has sometimes been raised as to whether there is any difference in the results obtained when livers are sampled frozen as compared to when they are sampled "fresh". Using soupfin shark livers, this was tested in two instances: once with 17 cans, and once with 84 cans. The livers were first sampled before freezing and then resampled after they had been frozen. As can be seen in Table IV in neither case did the dif-

Table II. Precision in Sampling 100 Cans of Fresh Soupfin Shark Livers

(One core taken per can)

Sample No.	Oil Content, %	Deviation of Oil Content from Average, Relative %	Oil Potency, U.S.P. Units Vitamin A per Gram	Deviation of Oil Potency from Average, Relative %	Liver Potency, Millions U.S.P. Units Vitamin A per Lb.	Deviation of Liver Potency from Average, Relative %
1	66.4	0.91	57,100	2.56	17.3	1.71
2	67.0	1.82	56,500	3.58	17.2	2.27
3	65.5	0.46	60,800	3.75	18.1	2.84
4	64.5	1.97	59,900	2.22	17.6	0.00
Av.	65.8	1.29	58,600	3.03	17.6	1.71

Table III. Precision of Samples of 35 Cans of Soft-Frozen Tuna Livers

(One core taken per can)

Sample No.	Oil Content, %	Deviation of Oil Content from Average, Relative %	Oil Potency, U.S.P. Units Vitamin A per Gram	Deviation of Oil Potency from Average, Relative %	Liver Potency, Millions U.S.P. Units Vitamin A per Lb.	Deviation of Liver Potency from Average, Relative %
1	26.0	0.38	6,500	4.33	0.77	4.05
2	26.5	1.53	6,230	0.00	0.75	1.35
3	26.3	0.77	6,360	2.09	0.76	2.70
4	25.8	1.15	6,070	2.57	0.71	4.05
5	26.1	0.00	6,010	3.53	0.71	4.05
Av.	26.1	0.77	6,230	2.50	0.74	3.25

Table IV. Results Obtained in Sampling Fresh and Frozen Soupfin Shark Livers

Total Cans in Lot	Oil Content, %		Oil Potency, U.S.P. Units Vitamin A per Gram		Liver Potency, Millions U.S.P. Units Vitamin A per Lb.	
	Fresh	Frozen	Fresh	Frozen	Fresh	Frozen
17	62.3	64.0	116,000	114,000	32.8	33.1
84	61.2	61.8	97,000	97,600	27.0	27.4

Table V. Accuracy of Samples of 100 Cans of Fresh Soupfin Shark Livers

(One core taken per can)

Sample No.	Oil Content, %	Deviation Oil Content of Core Sample from Ground Sample, Relative %	Oil Potency, U.S.P. Units Vitamin A per Gram	Deviation of Oil Potency of Core Sample from Ground Sample, Relative %	Liver Potency, Millions U.S.P. Units Vitamin A per Lb.	Deviation of Liver Potency of Core Sample from Ground Sample, Relative %
1	66.4	3.4	57,100	4.8	17.3	1.1
2	67.0	4.4	56,500	5.8	17.2	1.7
3	65.5	2.0	60,800	1.3	18.1	3.4
4	64.5	0.5	59,900	0.2	17.6	0.6
Ground sample	64.2		60,000		17.5	

Table VI. Accuracy of a Sample of 16 Cans of Fresh Grayfish Livers

(5 cores taken per can)

	Oil Content, %	Oil Potency, U.S.P. Units Vitamin A per Gram	Liver Potency, Millions U.S.P. Units Vitamin A per Lb.
Ground sample	72.7	14,200	4.67
Core sample	73.2	13,500	4.48
Relative % deviation of core from ground sample	0.69	4.92	4.07

Table VII. Precision of Samples of 55 Cans of Fresh Soupfin Shark Livers Taken with Sampler

(3/8-inch and 9/16-inch augers)

Sample No.	Diameter of Auger, Inches	Cores per Can	Wt. of Sample, Lb.	Sampling Time, Min.	Oil Content, %	Deviation of Oil Content from Average, Relative %	Oil Potency, U.S.P. Units Vitamin A per Gram	Deviation of Oil Potency from Average, Relative %	Liver Potency, Millions U.S.P. Units Vitamin A per Lb.	Deviation of Liver Potency from Average, Relative %
1	3/8	1	0.79	10	63.7	0.31	74,500	3.76	21.6	3.85
2	3/8	5	3.66	43	63.1	1.25	71,400	0.55	20.5	1.44
3	9/16	1	2.95	10	64.4	0.78	70,700	1.53	20.7	0.48
4	9/16	5	7.86	36	64.2	0.47	70,500	1.81	20.5	1.44
Av.					63.9	0.70	71,800	1.91	20.8	1.80

Table VIII. Precision of Samples of Grayfish Livers

(One core per can taken by sampler with 9/16-inch auger)

Total Cans in Lot	Sample No.	Sampling Time, Min.	Oil Content, %	Deviation of Oil Content from Average, Relative %	Oil Potency, U.S.P. Units Vitamin A per Gram	Deviation of Oil Potency from Average, Relative %	Liver Potency, Millions U.S.P. Units Vitamin A per Lb.	Deviation of Liver Potency from Average, Relative %
78	1	8	70.4	0.14	12,800	0.79	4.09	1.24
	2	8	70.0	0.43	13,000	2.36	4.13	2.23
	3	10	70.6	0.43	12,300	3.15	3.94	2.48
	4	11	70.0	0.43	12,400	2.36	3.94	2.48
	5	13	70.4	0.14	12,800	0.79	4.09	1.24
Av.			70.3	0.31	12,700	1.89	4.04	1.93
105[a]	1	..	71.1	1.57	15,100	2.58	4.88	0.81
	2	..	70.2	0.29	15,400	0.65	4.91	0.20
	3	..	69.1	1.29	15,700	1.29	4.92	0.00
	4	..	69.4	0.85	15,800	1.94	4.98	1.22
Av.			70.0	1.00	15,500	1.62	4.92	0.56
255	1	6	71.0	2.16	8,350	1.53	2.70	1.12
	2	8	70.2	1.00	8,600	1.42	2.74	2.62
	3	15	68.1	2.01	8,800	3.77	2.72	1.87
	4	20	68.7	1.15	8,500	0.24	2.64	1.12
	5	23	69.3	0.29	8,150	3.89	2.57	3.75
Av.			69.5	1.32	8,480	2.17	2.67	2.10
256	1	20	61.3	0.49	9.140	2.01	2.54	1.20
	2	34	62.3	1.14	8,850	1.23	2.50	0.40
	3	45	61.3	0.49	8,890	0.78	2.48	1.20
Av.			61.6	0.71	8,960	1.34	2.51	0.93
Av.				0.84		1.75		1.38

[a] These livers were soft-frozen. Other values in table are for fresh livers.

ference in the average results obtained by sampling the livers in the two states exceed 2%.

In the study of liver sampling, the primary object had been to develop a method that would give reproducible assays of the vitamin A content. If it were later found that the absolute vitamin content was not given by the analytical method, and that the method was uniformly biased in one direction, a correction factor could be applied. In order to determine whether or not such a bias existed, the accuracy of the sampler was tested twice, once with soupfin shark and once with grayfish livers. In these tests, samples were first taken with the corer, and then the remains of the livers were ground. Three separate ground samples were taken in each case to test their homogeneity. The three ground samples from each lot gave equivalent assays well within the limits (2%) of the method of analysis. Therefore, any deviation of the assay of the core sample from that of the ground sample can be regarded as evidence of a defect in the core-sampling method.

The results of these two tests, recorded in Tables V and VI, indicate that the core samples may be slightly high as regards oil content and slightly low as regards oil potency. Further tests would have to be made to establish this point. Fortunately, these two discrepancies tend to neutralize each other in the calculation of the liver potencies, and if any bias exists, it is of small magnitude.

In many cases, considerable free oil is to be found in the containers, especially if the livers have been long removed from the fish or have been frozen and subsequently thawed. Free oil was encountered in the case of the 84 cans of soupfin shark livers recorded in Table IV. These had been frozen and thawed, and the livers were buoyed up with oil. Nevertheless, the average results given by sampling the thawed livers were the same as those obtained after they had been refrozen. The analyses reported in Table V also were made on a lot containing appreciable free oil, yet the results obtained by core sampling were not greatly different from those obtained with ground samples.

Although the experience with frozen livers is limited, sufficient work has been done to show that the sampler will be a welcome addition in this field also. Under present-day conditions, freezing facilities are difficult to obtain, and the livers are often held at temperatures too high for hard-freezing. Consequently, there has been a real need for a device which will sample livers in the soft-frozen condition. The electric drills that are equipped for holding drill shanks up to 0.25 inch in diameter generally do not have sufficient power for use in sampling soft-frozen livers. However, those electric drills that are equipped for taking 0.5-inch shanks are able to handle the greater torque encountered with the frozen material. A skilled machinist can without difficulty make this change-over from the setup recommended for sampling fresh livers.

The standard sampler, employing a 1.125-inch auger, as described by McKee, Sanford, and Bucher (*1*), was used in the above tests. Smaller augers of 0.375-inch and 0.563-inch diameter had been tried in earlier experiments (*4*), and, for the information of those who might consider use of such modified equipment, results of the trials are presented in Tables VII and VIII. Precision was satisfactory, although clogging of the tube made frequent cleaning of the auger necessary.

The results summarized in Table VIII demonstrate that wide variations in the rate of sampling do not appreciably affect precision. These data also show the extreme rapidity with which the sampling can be carried out. Ordinarily, 100 cores can be taken within 15 minutes, so that more time is used for the incidental operations of removing and replacing the can lids than for the actual sampling. The ease of use and the satisfactory results obtained have led to the adoption of the device by many firms engaged in the fish liver trade.

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Colorimetric Determination of *p,p'*-DDT in Technical DDT

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A colorimetric procedure for the determination of *p,p'*-DDT is based on the color produced when this compound is heated in a mixture of glacial acetic and concentrated sulfuric acids. The method is applicable to the estimation of *p,p'*-DDT in mixtures with the *o,p'*-isomer and in technical DDT when a small empirical correction is made.

OF THE several analytical procedures described for DDT (*1*) only three (*2*, *3*, *4*) are suited for analysis of *p,p'*-DDT in technical DDT and these are macroprocedures. The present method, based on the formation of a yellow color when *p,p'*-DDT is heated in a mixture of concentrated sulfuric and glacial acetic acids, provides a simple, rapid means of determining 0.05 to 0.5 mg. of this compound with an accuracy of ±1%. The procedure described has been applied to the analysis of synthetic mixtures of *p,p'*-DDT and *o,p'*-DDT (1-trichloro-2-*o*-chlorophenyl-2-*p*-chlorophenylethane) and to several commercial samples of technical DDT.

It is believed that the colored product formed in this test occurs by oxidation of the DDT to the carbinol by the hot sulfuric acid. Aromatic carbinols are known to give colored solutions in sulfuric acid (*5*).

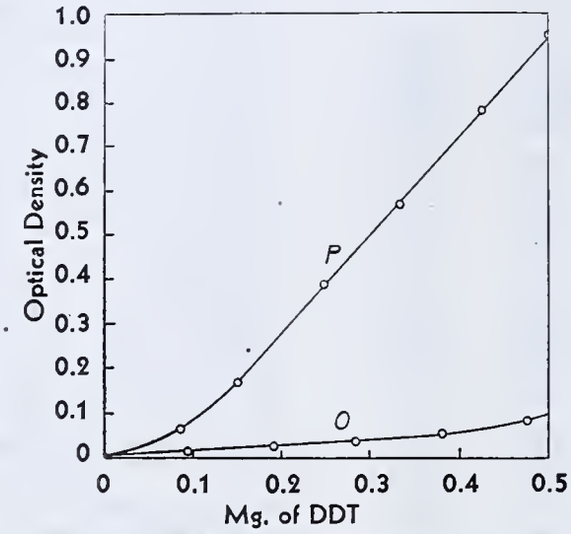

Figure 1. Calibration Curve for *p,p'*-DDT (*P*) and *o,p'*-DDT (O) at 435 mμ

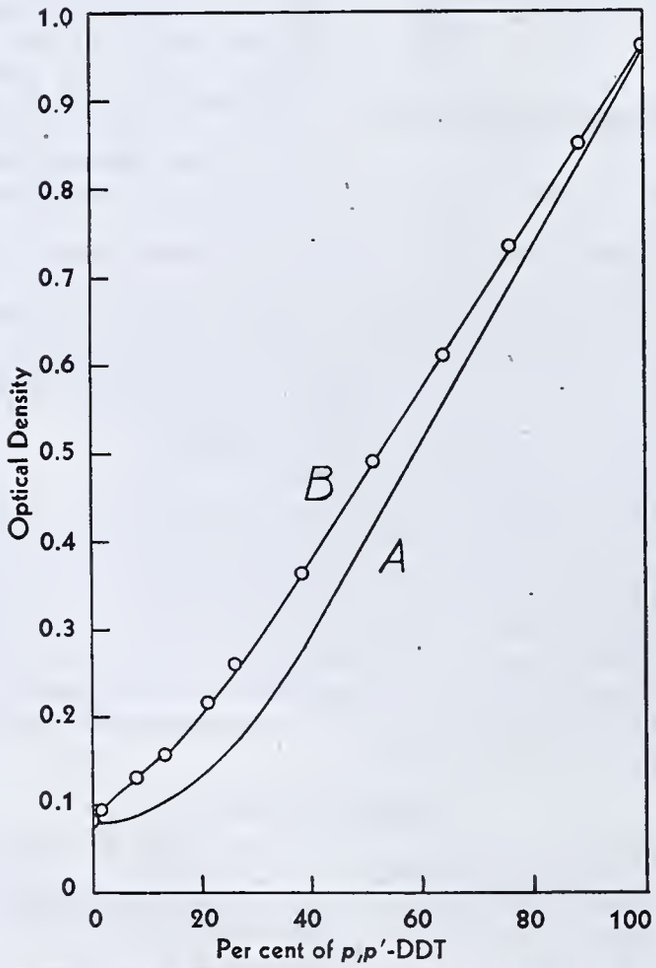

Figure 2. Calibration Curve for Mixtures of *p,p'*-DDT and *o,p'*-DDT at 435 mμ

A. Theoretical curve calculated by addition from absorption of separate solutions of *p,p'*-DDT and *o,p'*-DDT
B. Observed values

APPARATUS

The Beckman photoelectric quartz spectrophotometer, Model DU, was used with absorption cells of 1.00-cm. optical depth. Unless otherwise indicated, the measurements were made at 435 mμ using a slit width of 0.04 mm. The instrument was calibrated against colored solutions of known maximum absorption.

PROCEDURE

Weigh 0.06 gram of sample into a 250-ml. volumetric flask, dissolve, and dilute to 250 ml. with glacial acetic acid (1 ml. = ca. 0.25 mg. of sample). Using a pipet, transfer 2 ml. to a 25 ×

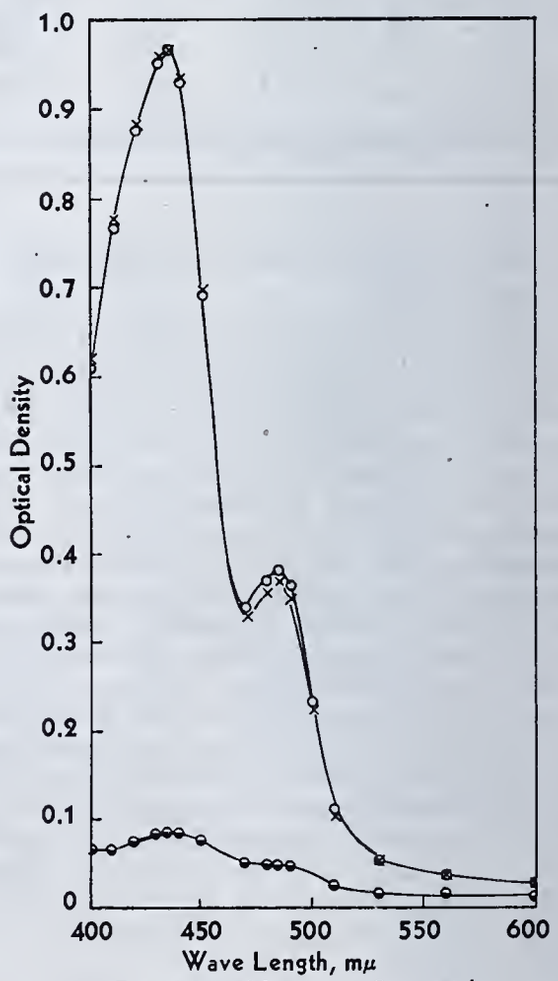

Figure 3. Absorption Spectra

○. *p,p'*-DDT, 0.500 mg. per 2 ml. of HOAc and 10 ml. of H_2SO_4
◒. *o,p'*-DDT, 0.477 mg. per 2 ml. of HOAc and 10 ml. of H_2SO_4
×. Technical DDT, 0.500 mg. per 2 ml. of HOAc and 10 ml. of H_2SO_4
Density values for technical DDT multiplied by a constant

Table I. Analysis of Samples of Technical DDT

Sample No.	Melting Range	Analyzed	Optical Density Observed	Optical Density Corrected	p,p'-DDT Found
	° C.	Mg.			%
1	81–99	0.499	0.715		
		0.499	0.710		
		0.499	0.720	0.716	74.1
			Av. 0.715		
2	80–97	0.500	0.720		
		0.500	0.715		
		0.500	0.720	0.718	74.5
			Av. 0.718		
3	78–94	0.519	0.720		
		0.519	0.730		
		0.519	0.720	0.698	72.3
			Av. 0.723		

200 mm. test tube. Add 10 ml. of concentrated sulfuric acid, C.P. Mix the contents of the tube, cool to room temperature, then immerse in a vigorously boiling water bath for 10 minutes. Cool, transfer sufficient solution to a spectrophotometer cell, and determine the optical density at 435 mμ.

The concentration of glacial acetic acid is critical; 1 ml. resulted in the development of 20% more color, 3 ml. in the development of 80% less color. A stable and maximal color was obtained with p,p'-DDT when the mixture was heated for from 9 to 14 minutes. After cooling the sample this color is stable for at least one hour.

Calibration curves for p,p'-DDT and o,p'-DDT and for synthetic mixtures of the two were obtained using aliquots of standard solutions made up to 2 ml. with glacial acetic acid and carried through the procedure described above. The melting ranges of the samples of isomers used were: p,p'-DDT, 107–107.5°; o,p'-DDT, 73.5–74°.

RESULTS AND DISCUSSION

The intensity of the color produced by p,p'-DDT is much greater than with o,p'-DDT (Figure 1). For analytical purposes the influence of o,p'-DDT is, however, not negligible, since mixtures of the two isomers show greater absorption than the sum of the absorption of the separate components (Figure 2). In the analysis of mixtures it is necessary, therefore, to use the experimentally determined calibration curve.

The absorption curve of the colored solution produced by this reaction from technical DDT is similar to that of p,p'-DDT (Figure 3). In fact, it may be brought into near coincidence by multiplication by a factor throughout. This factor is not necessarily the reciprocal of p,p'-DDT concentration because of the enhancement of color by o,p'-DDT as referred to above. More accurate results are obtainable by taking this effect into account.

The procedure for estimating the p,p'-DDT content in technical DDT is, then, the following: The optical density measured by analysis of a sample of technical DDT is multiplied by the factor $\frac{0.50}{\text{mg. analyzed}}$, which gives the optical density that would be obtained if the weight of the sample analyzed were 0.50 mg. The per cent p,p'-DDT is then read from Figure 2, B. The error incurred in the calculation by neglecting the impurities (assumed inert) other than o,p'-DDT is well within the experimental error.

In Table I are given the results of analyses of three commercial samples of technical DDT.

ACKNOWLEDGMENT

The sample of o,p'-DDT and one sample of technical DDT were kindly supplied by Weldon G. Brown.

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THIS work was carried out under contract with the Medical Division of the Chemical Warfare Service.

Semimicrodetermination of Saponification Equivalent by Rieman's Double-Indicator Method

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A method is described for the determination of the saponification equivalent with 5- to 100-mg. samples for saponification equivalents from 100 to 1000.

WITH some modifications, Rieman's double-indicator macromethod (*4*) for the determination of saponification equivalent has been adapted to a semimicroprocedure in accordance with his suggestion that it should be more easily adapted to a microprocedure than the standard method.

The procedure of Marcali and Rieman (*3*) is limited to compounds which are readily soluble in alcohol and which can easily be saponified. From 3- to 8-mg. samples, with saponification equivalents of about 200 to 300, are saponified in 2 drops of alcoholic potassium hydroxide; in the modified method 5 ml. of 0.5 N potassium hydroxide are used.

The modified method is especially useful on compounds that are difficult to dissolve in ethyl alcohol. Its accuracy is within 5% of the theoretical on pure samples of esters as well as ketones that will completely open the ring in the presence of alkali (Table I).

REAGENTS

0.5 N potassium hydroxide (approximate) in absolute or 95% ethyl alcohol (*2*).
Ethyl alcohol.
Distilled water.
Phenolphthalein in ethyl alcohol, 1%.
0.5 N hydrochloric acid (approximate).
0.025 N hydrochloric acid (standardized).
0.010 M bromophenol blue [1.3 grams of bromophenol blue and 2.0 millimoles (80 mg.) of sodium hydroxide, diluted with distilled water to 200 ml.].

APPARATUS

The saponification flask may be made from a 20 × 150 mm. Pyrex test tube. The flask, graduated at 5.0 and 10.0 ml., has a 19/38 standard taper female joint.

A Liebig condenser, 15 to 20 cm. in length, is connected to the saponification flask by a 19/38 standard taper male joint.

Table I. Comparison of Results by Macro- and Semimicromethods

Compound Analyzed	Saponification Equivalent: Macro	Calculated	Semimicro (modified method)
Ethyl iodophenyl undecylate	412[a]	416	408
Linseed oil	284[a]	285 (approx.)	280
Soybean oil	275[a]	285 (approx.)	278
	275[b]		
3a,4,7,7a - Tetrahydro - 2,3,-5,6 - tetraphenyl - 4,7-methanoindene-1,8-dione[c]	460[d]	464	455
α - 1,2,4,5,6 - Pentaphenyldicyclo - (2,2,1) 5 - heptene-7-one[c]	490[d]	506	500
Ethyl acetoxy propionate[e]	160[a]	160	104
β-Acetoxy ethyl lactate[e]	179[a]	179	120

[a] Standard method.
[b] Double-indicator method (macro).
[c] Ketones.
[d] Potentiometric method.
[e] Acetylated esters.

An Ascarite tube is connected to the end of the condenser by means of a U-tube and rubber tubing.

A 25 × 150 mm. hydrometer cylinder (or test tube) is used as the titrating vessel.

A 10-ml. buret graduated in 0.05 ml. is used for the titrations.

PROCEDURE

By means of a 12-cm. weighing tube the sample is weighed by difference into the saponification flask, ±0.3 ml. of 0.5 *N* alcoholic potassium hydroxide is added up to the 5-ml. graduation mark (10 ml. may be used if the sample is too difficult to dissolve). The flask is immediately connected to the condenser, which has the Ascarite or Caroxite tube attached. The mixture is refluxed from 0.5 to 3 hours.

After saponification, the condenser is rinsed with 1 to 2 ml. of alcohol and the flask disconnected. Five drops of phenolphthalein are immediately added and the solution is slightly acidified with 0.5 *N* hydrochloric acid. The mixture, which has about 0.25 ml. of 0.5 *N* hydrochloric acid in excess, is rinsed with ethyl alcohol into a 25 × 150 mm. hydrometer cylinder (or test tube). The total volume should be about 10 to 15 ml.; 0.5 *N* potassium hydroxide is added until the solution is slightly pink. The solution is then made just colorless with an unmeasured amount of 0.025 *N* hydrochloric acid.

Three drops (0.3 ml.) of bromophenol blue are added and the resulting blue solution is titrated with the standardized 0.025 *N* hydrochloric acid until a green color appears. The first color change is taken as the end point, which is blue-green. With a little practice, this end point can be reproduced to within 0.02 ml. of 0.025 *N* hydrochloric acid.

A blank of from 0.2 to 1 ml. of 0.025 *N* hydrochloric acid should be run under identical conditions. This variation is due to the impurities in the reagents, the volume of potassium hydroxide, and the length of the reflux period. If the volume of potassium hydroxide is measured within 0.5 ml., the blanks will be constant within 0.05 ml. of 0.025 *N* hydrochloric acid. The blank is the amount of 0.025 *N* hydrochloric acid added to change the bromophenol blue solution from blue to blue-green when no soap is present.

Precautions. The saponified solution is transferred (after acidifying) to a 25 × 150 mm. cylinder because small amounts of 0.5 *N* potassium hydroxide cling to the etched walls of the flask. If the solution were not transferred, this small amount of 0.5 *N* potassium hydroxide in the etched walls of the flask would only slowly be neutralized by the acid and would interfere in the titration of the soap with 0.025 *N* hydrochloric acid.

DISCUSSION

According to Rieman (*4*) the sharpness of the end point is increased with a decrease in the volume of the solution to be titrated. Because several organic compounds are difficult to dissolve in ethyl alcohol, the titrating volume of the modified method cannot be reduced. However, in most cases, the accuracy is sufficient to identify the compound.

Table II. Precision of Double-Indicator Semimicromethod

Compound Analyzed	Saponification Equivalent Found	Length of Reflux Period, Hours
Ethyl iodophenyl undecylate	404	1
	405	1
	410	1
	416	3
	405	3
Ethyl acetoxy propionate	100	1
	105	1
	103	3
α-1,2,4,5,6-Pentaphenyldicyclo-(2,2,1,)-5-heptene-7-one	495	3
	497	3
	508	2
	505	2
	495	3

In this laboratory, no trouble was encountered with the formation of fatty acids, probably because of the small amount of sample and the relatively large volume. The semimicromodification gives low results on acetylated esters (Table I). The precision of the method is given in Table II.

The fourth and fifth samples in Table I will be explained in a paper by C. F. H. Allen in *Journal of the American Chemical Society*.

Calculations (*1*)

Saponification equivalent = *SE*

$$SE = \frac{\text{mg. of sample}}{(\text{ml. of } 0.025\ N\ \text{HCl} - \text{ml. of HCl blank})\ (\text{exact normality of HCl})}$$

$$SE = \frac{\text{mg. of sample}}{\text{ml. of } 1\ N\ \text{KOH required to saponify}}$$

The volume of hydrochloric acid used in the calculation is the amount added between the phenolphthalein and bromophenol blue end points. It is equivalent to the volume of potassium hydroxide required to saponify the sample.

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Communication 1058 from Kodak Research Laboratories.

NOTES ON ANALYTICAL PROCEDURES

Isopropyl Alcohol in Cotton Wax Determination

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THE Conrad technique (*2*) for determining wax in cotton fiber was found more efficient and complete, within a reasonable extraction period, than any other technique tried. This method, long used at the Southern Regional Research Laboratory employs ethyl alcohol as the extracting solvent. The substitution of isopropyl alcohol as the extracting solvent is the subject of the present report.

Isopropyl alcohol, which has many of the properties of ethyl alcohol, is plentiful, readily available, cheaper than even denatured ethyl alcohol (*1*), and free from federal tax and accompanying restrictions. Moreover, it yields identical results when substituted for ethyl alcohol in the Conrad technique. Proof that this substitution can be made without sacrificing accuracy should, therefore, increase the usefulness of this excellent analytical procedure.

EXPERIMENTAL

In the application of the Conrad technique in the following experiments, the alcohols used throughout were the purified azeotropic water-alcohol mixtures, 95% ethyl alcohol distilled from potassium hydroxide, and 91% isopropyl alcohol decanted from sodium hydroxide and then distilled. To facilitate the phase separations, 100 ml. of water were used instead of the 75 ml. recommended in the original Conrad procedure.

Wax determinations were made on a series of cotton bagging materials using 95% ethyl alcohol and then repeated, substituting 91% isopropyl alcohol as the extracting medium. The procedures were identical in all other respects.

The results (Table I) show that isopropyl alcohol extracted practically the same percentages of the waxy substances as the ethyl alcohol, the small differences observed being within the usual limits of experimental error.

Experiments in which the quantities of alcoholic extract from identical cotton fabrics were given as a function of time (Table II) showed that no difference existed between ethyl and isopropyl alcohols in extraction time. Isopropyl alcohol extracts as rapidly as ethyl alcohol and, when the same techniques are used, yields the same quantities of wax. Moreover, the general appearance of the two extracts was identical, and no differences were detected in the properties of the extracted fabrics.

Table I. Wax Values by Conrad Method

Series No.	Treatments	Wax Values: Ethyl alcohol solvent	Wax Values: Isopropyl alcohol solvent
		%	%
439	Gray goods, control	0.72	0.71
440	Kier boiled and soap washed	0.74	0.74
444	Kier boiled	0.60	0.64
445	Kier boiled, soured	0.53	0.54
446	Kiered, soured, H_2O_2 bleach	0.36	0.33
447	Kiered, soured, chlorine bleach antichlor	0.40	0.40
448	Kiered, soured, chlorine bleach, sour	0.41	0.41

Table II. Speed of Extraction of Wax from Cotton by Isopropyl and Ethyl Alcohol

Extraction Time	Wax Values: Isopropyl alcohol	Wax Values: Ethyl alcohol
Hours	%	%
2	0.83	0.79
4	0.81	0.87
6	0.85	0.84
7	0.87	0.85
8	0.86	0.85

Table III. Sugars Transferred to Chloroform by Ethyl and Isopropyl Alcohol by Distribution from Solutions Containing 1 Gram of Sugar

Sugar Taken	Isopropyl Alcohol	Ethyl Alcohol
	Mg.	*Mg.*
Sucrose	0.0	0.8
	0.1	0.8
Dextrose	0.0	0.0
	0.0	0.0
Levulose	0.0	2.4
	0.1	1.6
Blank determination	0.3	0.0
	0.0	0.0

Conrad (*2*) has pointed out that no appreciable quantities of the sugars occurring in cotton fibers, such as perhaps sucrose, dextrose, or levulose, are transferred from ethyl alcohol to the chloroform with the wax. To compare the relative quantities of sugars transferred to chloroform by ethyl alcohol and by isopropyl alcohol, duplicate 1-gram samples of each sugar were dissolved in 100-ml. quantities of isopropyl alcohol and in like quantities of ethyl alcohol in the separatory funnels, chloroform and water were added, and the determination was completed in the same way as for wax. Table III shows that isopropyl alcohol, probably because it is a better solvent for sugars than ethyl alcohol, carried all the sugars into the watery layer, allowing none to be transferred to the chloroform. In this respect, isopropyl alcohol seemed superior to ethyl alcohol as a solvent in wax determinations. The differences, however, are not significant.

Soap, when present, is determined as wax by the Conrad technique. This characteristic is not changed by substitution of isopropyl for ethyl alcohol.

CONCLUSIONS

The substitution of isopropyl for ethyl alcohol as the extracting solvent in the Conrad method for the determination of wax in cotton fiber does not alter the values obtained. Extraction with isopropyl alcohol is accomplished as rapidly and completely as with ethyl alcohol, with no apparent damage to the resulting fabrics. The general characteristics of the extracts obtained with both solvents appear to be identical; and both solvents eliminate the transfer of sugars to the isolated wax.

ACKNOWLEDGMENT

Appreciation is expressed to Carl M. Conrad for helpful criticisms and advice on the work here presented.

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Preparation of Silica Gel for Chromatography

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DURING the past 2 years the authors have prepared for the chromatography of penicillin considerable quantities of silica gel. This method of preparation is a modification of that reported by Gordon, Martin, and Synge (*1*) and described in the experimental section of their paper, "Partition Chromatography in the Study of Protein Constituents".

Silica gel which is satisfactory for partition chromatography is difficult to prepare under the best of conditions. Since it is likely to be more widely used in the future, the method of preparation is reported in detail.

PROCEDURE. In a 115-liter, glass-lined tank, 34 liters of sodium silicate (Merck's sodium silicate solution, sp. gr. 1.38 to 1.40, Bé. 40° to 42°) and 10 liters of water are mixed with a high-speed stirrer (Lightnin air mixer, Type AR-25, 1750 r.p.m., 0.25 h.p., two 7.6-cm. propellers). Hydrochloric acid (10 *N*) is added very slowly (17 liters in 2.5 to 3 hours) with vigorous stirring until the mixture is permanently acid to thymol blue (pH 2.0 to 2.8). A large separatory funnel is convenient for the addition of the acid.

When about 4 liters of the hydrochloric acid have been added, a very thick, gummy mass is formed. At this point the addition of acid is stopped, and the mass is thoroughly broken up with a heavy wooden paddle. More acid is added dropwise with stirring until a thin suspension results. The rate of the addition of acid can then be increased until the desired pH is reached.

The suspension is allowed to digest at 25° C. with continuous stirring for 2 hours, then filtered by suction on a large stoneware filter (66-cm. diameter). Sharkskin paper (Carl Schleicher and Schull Co., 116 West 14th St., New York, N. Y.) plus a thick filter pad has been used for this filtration. A hard cake of very fine silica gel forms on top of the filtering material. This must be broken up in order to speed the filtering process which usually requires about 14 hours. At this point the silica gel can be aged in 0.2 *N* hydrochloric acid as described by Synge (*1*). This requires 2 days and increases the buffer-adsorbing titer of the product by approximately 20 to 40%.

In either case the silica gel is suspended in tap water, filtered, and washed until free of acid. The washed silica gel is dried at 200° C. for 12 hours and ground to a particle size of 50- to 150-mesh, using a Fitzpatrick Model D hammer mill. The ground silica gel is washed with distilled water until free of chloride (silver nitrate test) and dried in shallow pans at 250° C. for 24 to 48 hours. This time and temperature are necessary in order to obtain a product having a maximum buffer-adsorbing titer. Silica gel prepared without aging in 0.2 *N* hydrochloric acid adsorbs 85 to 100% of its weight of water without becoming lumpy or moist. For purifying penicillin the less expensive unaged product has been found satisfactory.

The final product is transferred to dry bottles while still hot and sealed to avoid moisture uptake. From 34 liters of sodium silicate solution approximately 15 kg. of 50- to 150-mesh silica gel are obtained. This is enough for the chromatography of approximately 100,000,000 units of Penicillin G.

The isolation of pure penicillins using silica gel chromatography will be reported in a separate publication.

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Determination of Small Amounts of 4-Vinyl-1-cyclohexene (Butadiene Dimer) in Tetrahydrophthalic Anhydride

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TETRAHYDROPHTHALIC anhydride (4-cyclohexene-1,2-dicarboxylic acid anhydride) is usually prepared by the Diels-Alder reaction (*1*) of 1,3-butadiene with maleic anhydride. The product, which is obtained in practically quantitative yield, is pure except for the presence of minute amounts of the dimer of butadiene, 4-vinyl-1-cyclohexene, which is formed at the temperature needed to keep the maleic anhydride liquid for ready reaction—e.g., 80° to 100° C. From the viewpoint of control and constancy of product, it was desirable to have a rapid, simple method for determining fractions of a per cent of the butadiene dimer in the tetrahydrophthalic anhydride. A solution was found in the selective extraction of the vinylcyclohexene from aqueous alkaline solution by cyclohexane and in the determination of the vinylcyclohexene in the cyclohexane extract by the determination of the extent of unsaturation.

Subsequent to the work described in this report a method for the determination of vinylcyclohexene in recycle styrene was described by Laitinen, O'Brien, and Wawzonek (*2*). The sample is dissolved in dioxane and the styrene polymerized on refluxing, using sodium as catalyst; the vinylcyclohexene left in solution is determined by the iodine chloride method. The method is sensitive to 0.2% vinylcyclohexene and is accurate to ±0.2% (absolute) over a range of 1 to 20% vinylcyclohexene. Since this method involves removal of the principal constituent by selective polymerization, it could not be applied to the determination of vinylcyclohexene in tetrahydrophthalic anhydride; in addition, the sensitivity was insufficient for the range of concentration covered.

PROCEDURE. The sample size used is governed by the amount of vinylcyclohexene believed present. For the range of 0.01 to 0.10% a 10-gram sample, weighed to the second decimal place, of product tetrahydrophthalic anhydride is added to a 250-ml. separatory funnel containing 40 ml. of aqueous 10% sodium hydroxide solution, followed by the addition of 50 ml. of cyclohexane (free of unsaturated material). After agitation and separation into layers, the lower aqueous layer is removed and the extraction of the cyclohexane fraction is repeated. Three extractions are sufficient. The extent of unsaturation of the cyclohexane extract can be satisfactorily determined by the bromide-bromate titration method of Mulliken and Wakeman (*3*). The percentage of vinylcyclohexene in the sample is readily calculated from the bromine number found, 296 being the bromine number for pure vinylcyclohexene, or from the milliequivalents of bromine consumed, 27.05 being the equivalent weight of vinylcyclohexene.

When the procedure was applied to samples of vinylcyclohexene-free tetrahydrophthalic anhydride (bromine number = 105), the cyclohexane extract had a bromine number of 0.00. Application of the procedure to small samples of vinylcyclohexene showed quantitative recovery within 10 relative per cent. Analysis of synthetic mixtures of vinylcyclohexene and tetrahydrophthalic anhydride containing 0.02 to 2.0% of the former showed similar recovery. The vinylcyclohexene used in these experiments was obtained by careful fractionation of the dimer prepared by the liquid-phase thermal dimerization of butadiene. The physical constants of the material used checked the values reported in the literature. Analysis of a typical preparation of tetrahydrophthalic anhydride indicated 0.038 ± 0.0035% vinylcyclohexene to be present.

The determination of vinylcyclohexene by halogen addition her than by the method of Mulliken and Wakeman (*3*) is dis- ıssed by Laitinen and co-workers (*2*), who found that 93 to 97% the theoretical amount of halogen was added. The bromate ethod used in this report was found to give 94 ± 2% of the ılculated halogen addition, which is sufficiently accurate for the w concentration range covered. Application of a correction the type used by Laitinen and co-workers (*2*) would improve ıe accuracy of the results; for high concentrations of vinyl- ʏclohexane it would be preferable to use such a correction.

It is apparent that the procedure described can be applied to the determination of vinylcyclohexene in any saturated or unsaturated substance which is not extracted by cyclohexane from alkaline or neutral aqueous solution.

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Distilling Apparatus for Production of Pure Water

FREDERIC E. HOLMES, 6515 Blueridge Ave., Cincinnati 13, Ohio

THE apparatus shown in Figure 1 was developed and used in the Clinical Laboratory at Bushnell General Hospital (Army ervice Forces, Ninth Service Command, Brigham, Utah) to ıpply water of considerably higher purity than that ordinarily vailable, for preparation of gold sols, and for other special serolog- :al and chemical preparations and uses. It was not necessary to btain water of the extreme purity required for certain physical ıeasurements or for determining extremely minute amounts of arious substances. The assembly and operation of the still had o be of utmost simplicity, for use by personnel whose interest nd training were primarily in clinical methods, without diverting ttention from their primary work to the still during operation to void entrainment, flooding, or other faults in performance or to nake control tests of the distillate. The apparatus was designed o meet severe limitations of bench space and head room and of naterial available for construction.

The present still is a modification of one used at Wright Field *1*).

The output of batch operation (approximately 2 liters daily) vas adequate, making it possible to eliminate the reservoir and ıccessories for maintaining constant level of raw water in the boil- ng flask used at Wright Field. More elaborate accessories for control of flow of cooling water were eliminated by the "mixer", in which relatively large changes in rate of flow of cold water, obtainable by a crude valve such as a sink faucet, induce small changes in rate of flow of convection currents between mixer and condenser.

The column is held upright by a single clamp, with its weight resting on a ring of clean cotton in the bottom of the water seal. (This type of joint was used to reduce the danger of breakage because ball-and-socket ground joints were not available.) A Meker burner is adequate for heating. Rate of boiling and condensation are adjusted to waste about one third of the water vapor through the vent at the top.

A sample submitted to the Bureau of Standards was reported to contain nonvolatile electrolyte equivalent to 0.28 p.p.m. of sodium chloride by conductivity measurement, part of which may have been dissolved from the container in transit.

The same distillation may be performed in two steps in a column assembled with standard parts. The suggested column consists of a boiling flask, Hempel distilling column packed with short pieces of glass tubing in the lower half, take-off adapter with stopcock, and Liebig reflux condenser (Ace Glass, Inc., Catalog Nos. 5910, 5245, 6615, 6902, and 5030), all connected by ball-and-socket joints. Each batch of water is first refluxed with the stopcock closed to drive off gases and other volatile solutes. The stopcock is then opened for collection of the distillate. In this column less glass surface is exposed to action of hot water, and much of the soluble material from the surface of the glass which would otherwise contaminate the distillate is extracted during the first boiling and remains with the residue in the boiling flask during the distillation.

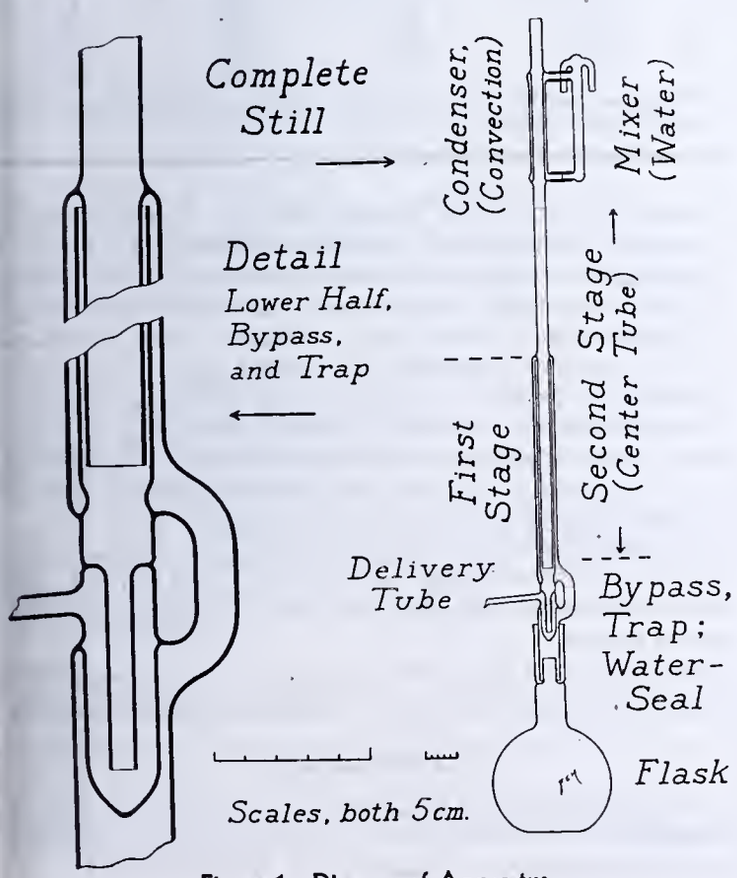

Figure 1. Diagram of Apparatus

When the trace of dissolved material from the glass can be considered negligible, the choice of columns becomes a matter of convenience and personal preference. The column used at Bushnell has some advantage in simplicity of operation. No packing is required to prevent entrainment. It is almost impossible for the apparatus to function improperly. No attention is required for change from a first to second stage of distillation, and, since both stages proceed simultaneously, only half the time is required for the same duration of reflux in the entire distillation. Such considerations may be of considerable importance in a busy laboratory where the still is easily forgotten.

The dimensions of the column are not critical, and can be estimated with sufficient accuracy by reference to Figure 1; 33-, 24-, and 19-mm. (outside diameter) tubing is satisfactory for outer (first-stage), middle (return), and inner (second-stage) tubes. A tube approximately 12 × 40 mm. is fused into the neck of a 2-liter flask to form the water seal.

ACKNOWLEDGMENT

The author wishes to thank George F. Liebig, Jr., for calling his attention to the advantages of the standard column, which is undoubtedly in common use by many chemists but not familiar to others.

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Regeneration of the Walden Silver Reductor

EUGENE H. HUFFMAN, Radiation Laboratory, University of California, Berkeley, Calif.

THE usual method for regeneration of the silver reductor is that given by the originators of the reductor (*1*). This method consists of filling the column with about 0.1 *M* sulfuric acid and allowing a zinc rod to remain in contact with the silver until the dark silver chloride has disappeared. Although effective, this method sometimes requires as much as 48 hours. When such reductors are subjected to considerable use, it may be necessary to have several of them undergoing reduction for each one in use. To avoid the necessity of having at least 12 reductors at hand, the following directions for regenerating the silver have been used in this laboratory:

Approximately 15 grams of crystalline chromic chloride are dissolved in 50 ml. of water in a 250-ml. Erlenmeyer flask and about 8 grams of granular zinc added. Concentrated hydrochloric acid is then added slowly until there is a brisk evolution of hydrogen, and more acid is added as the reaction subsides. When the reduction to chromous chloride is complete, as indicated by the blue color, the solution is poured into the reductor and allowed to percolate through the silver-silver chloride column. The solution is green for a while as it leaves the reductor. The reaction is complete when the solution comes out with a blue color. The chromous chloride solution is then rinsed from the reductor with 0.1 *M* sulfuric acid and the reductor is ready for use.

The total time required for the regeneration of a reductor which has been darkened for three quarters of the length of the silver column is 20 to 30 minutes, including the preparation of the chromous chloride solution. The reductors have the same characteristics after this treatment as after regeneration by zinc.

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New Type of Reflux Condenser

WILLARD T. SOMERVILLE, Elmhurst, N. Y.

WHEN a reflux condenser of conventional design is operated at or near its capacity, the friction between the ascending stream of vapor and the descending condensed film on the glass causes a reduction in the amount of return condensate and a holdup on the condensing surfaces. The condensed film is thickened and in a short time enough fluid is held back so that the space between the condensing walls is bridged with liquid. This results in "slugging" or "flooding", which becomes worse on the continued entrance of vapor into the condensing chamber. It is then necessary to stop the operation to permit the fluid to drain back into the reaction vessel. Often it is not desirable or possible to interrupt the refluxing, especially when exothermic reactions are encountered, to allow the trapped condensate to return. The present paper relates to a reflux condenser with increased capacity and a nonflooding feature.

The apparatus shown in Figure 1 (patent applied for) consists of a trap integrally constructed with an Allihn condenser; the upper part of the trap is cooled via the common wall. The trap has three or more openings in the floor, permitting the condensate to drain back to the reaction vessel. The path of the vapor, for the most part, is free of contact with returning condensate. When this apparatus is operated at or near capacity, the retained liquid is held in the trap and does not impair the efficiency of the condenser. If any fluid is held in the bottom of the trap, it will return to the vessel when the violence of the ebullition subsides. This condenser has been found very useful in controlling bumping and foaming liquids, since they are thrown against the side wall and can drain back.

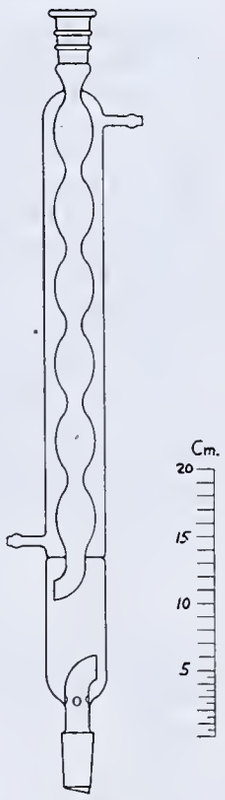

Figure 1

In Table I, data are given on an esterification using mineral acid as catalyst, and benzene as the azeotropic water-entraining substance. This reaction proceeded at a rate faster than the water could be removed, resulting in the formation of numerous large droplets of water in the reaction mixture. In a simultaneous experiment, a condenser constructed according to Figure 1 was compared with an Allihn condenser of the same dimensions, but without the trap. The reaction vessel was heated as strongly as possible without flooding the condenser. Table I indicates that the time for the completion of reactions of this type may be materially reduced by the use of this improved reflux condenser. The true differences between the two condensers are greater than shown, owing to the reduction in reaction rate as the concentration of reactants are reduced. The last few milliliters of water are particularly slow in formation and removal.

Table I. Esterification of Phenylacetic Acid with *n*-Pentanol

(Reaction mixture, 15 moles of acid, 20 moles of alcohol, 500 ml. of benzene, 5 ml. of H_2SO_4)

	Condenser of Figure 1		Allihn Condenser of Same Dimensions	
Time	Water in moisture trap used	Reaction water collected	Water in moisture trap used	Reaction water collected
Min.	*Ml.*	%	*Ml.*	%
0	0	0.0	0	0.0
15	35	12.4	32	10.8
25	48	17.0	42	14.9
45	96	33.9	84	29.8
55	124	44.8	108	38.3
70	151	53.3	131	46.4
90	214	75.6	186	66.9
105	259	91.5	229	81.3
135	276[a]	97.4	268	95.0
150	282	99.7	274[a]	97.2
175	283	100.0	281	99.7
215	...	...	282	100.0

[a] Water measured was hot, which may account for larger volume. Theoretical amount is 270 ml.

During this reaction, the condenser of Figure 1 was operated so that none of the condensate was held up in the trap; it could have been operated at a greater rate if some condensate had been held in this trap.

The above data show that a condenser of this design has a larger capacity and can cope with bumping and foaming liquids.

ACKNOWLEDGMENT

The cooperation of William Geyer in constructing several experimental models is appreciated. This condenser is available from the Scientific Glass Apparatus Co., Bloomfield, N. J.

INDUSTRIAL AND ENGINEERING CHEMISTRY
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Analytical Edition
WALTER J. MURPHY, EDITOR

Style Requirements

In April we discussed editorially the field which the Analytical Edition should endeavor to include in its coverage, emphasizing its desire to print more papers on evaluation of analytical results, statistical treatment of analytical data, principles and theory of analytical chemistry, and college training of personnel. The Analytical Edition is in position to print all such material promptly.

Manuscripts offered for publication differ widely, not only in scientific value but in their presentation. Some are well written, logically developed, concisely written, yet giving all necessary details. When these manuscripts are typed double space, with wide margins to allow room for directions to the printer, and are accompanied by drawings and photographs from which engravings can be made without retouching or relettering, they gladden the editor's heart.

Others, however, fail to meet these specifications, and it is to authors of such papers, as well as to those who have not previously contributed to our pages, that this editorial is directed.

To be of the greatest service the scientific facts must be presented in a clear, logical readable manner. An important point to be kept in mind in writing any article is the type of reader to be reached. Readers of the Analytical Edition are intelligent scientists, who do not need schoolbook directions for laboratory procedure; they do need to be given sufficiently explicit directions so that they can repeat the work reported, if need be.

When a scientist has work to report in a certain journal—the Analytical Edition or any other—he should familiarize himself with that journal and model his report on particularly good papers published there. A little time devoted to thinking the article through before putting pen to paper will pay dividends in producing a logical, concise, and readable presentation that avoids repetition yet gives all the facts. An outline might be set up, for instance, as (1) why the work was done, with a brief reference to previous work, properly documented, (2) a concise account of what was done, with all necessary details as to apparatus, reagents, and procedure followed, and (3) the result of the investigation and conclusions drawn.

A short abstract that can be printed at the beginning of the article is a great help to the busy reader, as it enables him to decide at once whether to read the article in full then and there, to lay it aside for later attention, or to skip it as outside his field of interest.

Too many manuscripts give the impression that the author has dictated a chronological account of what he has done in the laboratory or—worse—has asked his secretary to copy hand-written notes and then has failed to read the manuscript over and check it for accuracy. A good secretary will take care of misspelled words and punctuation, but cannot always interpret carelessly written figures or chemical terms. Footnotes are too often an indication of sloppy thinking. The material there given might better be incorporated in the text or perhaps presented in a separate note.

Literature citations should be carefully checked for accuracy and completeness. Industrial and Engineering Chemistry prints them at the end of each article, arranged alphabetically by authors, and with corresponding numbers inserted in the text at appropriate places. The abbreviations used are given in the "List of Periodicals Abstracted by *Chemical Abstracts*". The spelling and nomenclature approved by the Society's Committee on Nomenclature, Spelling, and Pronunciation are followed. This subject has been recently discussed in the introduction to the 1945 subject index to *Chemical Abstracts*. The Merriam Webster dictionary is followed as the authority for deciding on the spelling of nontechnical words.

Specifications and directions for preparing copy, including illustrations, are given in "Suggestions to Authors", copies of which will be sent on request to any who are interested. A summary is printed on page 340 of this issue.

Having a definite bearing on the question of the preparation of chemical papers is what seems to be a regrettable lack of cooperation between the departments of chemistry and of English in most of our colleges and universities. If the ability to write a clear, concise report is not developed during the college course, it is acquired only with difficulty after graduation. Is not this a subject that should be given greater consideration by our chemical educators?

Improvement of Precision by Repeated Measurements

Application to Analytical Control Methods

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This article critically examines replication of measurements as a means of increasing precision. Formulas are provided for evaluating such improvement of precision in any given case and for computing confidence limits in routine analysis.

IN A previous paper (*2*) it was shown how the estimation of the precision of analytical methods can be accomplished by means of modern statistical theory. This article considers in detail some questions arising in the study of precision.

While the practice of at least duplicating every analysis is well established among analysts, the increase in precision thus brought about has seldom been considered. However, this question appears to be of considerable importance, as can be inferred from the following considerations.

In the practice of routine analysis, it is customary to obtain some indication about the precision of analytical results from the "closeness" of the replicate determinations (generally duplicates) made by the analyst. In some cases the optimistic picture thus obtained is tempered by the knowledge, gained through experience, that this closeness is very often grossly misleading. Moran (*3*) has given some attention to this matter, and has studied it experimentally on the basis of data obtained in his laboratories. Having found that the ratio of the standard deviation of single determinations to that of averages of duplicates was 1.05—appreciably less than the theoretical value $\sqrt{2}$ for the case of true random determinations—Moran (*3*) ascribes this result to the fact that "duplicate analyses made at the same time are not truly random but are rather influenced by slight variations in technique or surrounding conditions".

In a population of variance σ^2, the variance of the mean of a random sample of n is σ^2/n; the corresponding standard deviation is thus $\sigma/\sqrt{n}$. The ratio mentioned above is, therefore, in the case $n = 2$, $\dfrac{\sigma}{\sigma/\sqrt{2}} = \sqrt{2} = 1.41 \ldots$

Moran calls this ratio the "ratio of improvement by duplication". It seems preferable to consider the ratio of the variances, the squares of the standard deviations; then the theoretical value for duplication is simply 2; for n-fold replication it will be n. The advantage lies in the intuitiveness of the interpretation. On the other hand, the standard deviations referred to in Moran's "ratio of improvement" are "population parameters"—i.e., fixed though unknown constants characterizing the distribution of errors in the case considered. Now these quantities being unknown, we substitute for them estimates derived from the data available; but such estimates are obviously subject to fluctuations or, in statistical terminology, to "sampling errors". Thus in considering the ratio 1.05 obtained by Moran, it is well to keep in mind that a repetition of the whole experiment might have given a quite different value, perhaps 1.23, which is already much closer to the number 1.41 based on the hypothesis of randomness. For this reason it becomes desirable to infer from the data available not only the estimated ratio, but also an evaluation of the precision with which it is obtained.

This matter, among other things, is discussed in the following pages; formulas are given, based on the theory of statistics, for determining various quantities which are of interest in this connection. The calculations involved in this type of analysis of data, which will be seen to be simple and on an elementary level, are described in Section III and illustrated in Section VI by means of the data given in Moran's paper (*3*). The reader is, however, strongly urged to give careful consideration to Section I, where the chemical situation is described to which the proposed method is applicable. It cannot be expected—as it never can be in the practical application of theoretical considerations—that the conditions of Section I will be exactly fulfilled. It is rather a matter of personal judgment on the part of the chemist to decide whether the conditions in his case correspond sufficiently to the theoretical model described to permit application of the present method. Such subjective judgment can efficiently be supplemented by statistical tests of randomness, of equality of precisions, and the like (cf. *10*). While these tests are valuable in that they offer means of evaluating in an objective and consistent way a variety of important aspects connected with the precision of analytical methods, the author feels that they will in general not be necessary for the application of the method proposed in this paper, provided the analytical procedure in question is well defined in its various steps. This point is brought out more clearly in the following discussion.

I. APPLICABILITY OF THE TEST

Suppose that in order to gain quantitative information about the behavior of an analytical procedure in routine work, the research analyst devises one of the following experiments:

1. A sample, containing ν% of the considered constituent, is stored and analyzed at regular intervals, say every month, for a period of M months, by the same chemist, in N-fold replication each month. The N replicate determinations are to be performed as closely together as they are expected to be made in actual routine work. Thus M series, of N parallel determinations each, are obtained.
2. A similar experiment is performed, involving several analysts, with the understanding, however, that (*a*) every series of N replicates is performed by one analyst only; and (*b*) a random experiment is performed to select the analyst for every series. Thus if 4 analysts are available, 4 slips of paper, one of which is marked, are thoroughly mixed, after which every chemist draws one slip; whoever draws the marked slip performs the series of analyses for this month, regardless of possible repetitions due to chance.
3. The type of experiment is modified, in that M laboratories are selected at random (in so far as this is feasible, considering their suitability for the given type of analysis, etc.). In this case it may be superfluous to space the M series in time: M samples of the same material are sent simultaneously to the various laboratories.
4. Any similar type of experiment can be considered, with the twofold restriction, however, that (*a*) only one analyst makes the N replicates in a given series; and (*b*) the series are more or less random with respect to the factor which is considered to cause marked discrepancies between series, such as laboratory, chemist, weather conditions, etc.

Under these conditions the following assumptions are made:

A. To every series, say the ith, corresponds a "population mean" μ_i (which may be interpreted as the mean of all the analyses, in infinite number, which could be performed under the same stability of conditions as prevailed for the N analyses actually performed in this series). As pointed out by Moran, one should not in general expect this μ_i to be equal to ν, even in the case of a very accurate method of analysis, since slight variations in the surrounding conditions will influence similarly all the results of the series.

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B. All series are performed with equal precision. This does not mean, of course, that the estimate of precision (*2*) is to be identical for all M series; it rather implies that no significant difference exists between these estimates. As pointed out previously, this assumption, the validity of which might be questioned by the reader, is believed by the author to hold in general, provided the analytical method under study is described in sufficient detail—i.e., to such an extent that no wide divergence in pH values, temperatures, volumes used, etc., can arise—and provided the analysts are all reasonably competent.

C. Within each series the various results are distributed according to the "normal" ("gaussian") law of errors (cf. any elementary textbook on statistics, e.g., *1*). There can hardly be a question that this is at least approximatively true for most analytical results.

D. The values μ_i themselves are considered as a random sample of a normally distributed population corresponding to the totality of laboratories in a given region, or to the totality of replicate series in a given laboratory, etc., according to the type of experiment as discussed above. The question whether ν is the true mean of all these μ_i is of no concern to us in this connection, since all we are interested in here is the precision of the method.

Let ν' represent the population mean value of all the μ_i and let ν be the "true value" (or the theoretical value in the case of a synthetic sample); if $\nu' = \nu$ the method is accurate; if $\nu' \neq \nu$, $\nu' - \nu$ is the systematic error of the method (cf. *2*).

Here again there may arise serious doubt in the reader's mind concerning the validity of this assumption. However, if one is willing not to generalize beyond reasonable limits the validity of the results obtained by the statistical analysis, there is really no great danger of going far astray by accepting these results. In most cases, common sense will tell where one should draw the limit.

II. PURPOSE OF THE TEST

Our first aim is to estimate what Moran calls the "ratio of improvement" by N-fold replication, or rather its square, which we shall call the "coefficient of improvement by N-fold replication" and denote by the symbol $[CI]_N$. Thus:

$$[CI]_N = \frac{\text{variance of a single determination}}{\text{variance of the average of } N \text{ parallel determinations}}$$

We cannot calculate this quantity, since it contains unknown parameters; therefore we will supplement our sample estimate of it, which we will denote by $Est[CI]_N$, by a lower limit, L_1, and an upper limit, L_2, such that we may have considerable confidence that the interval L_1L_2 includes the true value $[CI]_N$ (*2, 4, 7, 8*).

It is evident that the larger the variability between series, the lower will be $[CI]_N$. As a matter of fact, no amount of replication, however great, within a series, can compensate completely for the loss of precision due to the systematic error that affects the whole series. On the other hand, a single blank will completely eliminate this bias. (Blank is here taken to include the concept of a "control" run on a sample of known composition; the value of the blank is then the difference between the result obtained and the true value.) Therefore it will be of interest to consider two other quantities: (*a*) the coefficient of improvement due to infinite replication (within a series), denoted by $[CI]_\infty$; and (*b*) a coefficient of improvement obtained by running n replicates simultaneously with n' blanks and subtracting the average "blank" from the average "sample". Let us represent the latter coefficient of improvement, which like the others is taken with respect to the precision of a single determination, by the symbol $[CI]_n^{n'}$.

The best choice of n and n', for a given total $n + n'$ of analyses, depends on the ratio of precisions with which the blank and the sample, respectively, are determined: If it appears from the type of analysis that the absolute errors are of the same order of magnitude for sample and blank, the best allocation is $n = n'$. But if there is reason to believe that the absolute errors for the sample are c times as great as those for the blank, the highest precision is obtained by taking $n/n' = c$.

In order to evaluate the practical significance of the calculations described below, it is necessary to distinguish between the task of the research analyst and that of the routine chemist. While for the former any parameter describing the precision of his method will be of value, the latter, on the contrary, will be interested in any new concept only in so far as it may help him in locating in the best possible way the unknown value he is asked to determine by analysis. What contribution our statistical analysis can make to this end is being discussed in Section V. A mathematical justification of the statistical test procedure is given in Section VII.

III. STATISTICAL TEST PROCEDURE

Let there be M series of N replicates each. We can represent them as follows:

1st series: $x_{11}\ x_{12}\ x_{13} \ldots x_{1N}$
2d series: $x_{21}\ x_{22}\ x_{23} \ldots x_{2N}$
. . . . . .
. . . . . .
Mth series: $x_{M1}\ x_{M2}\ x_{M3} \ldots x_{MN}$

In general $x_{i\alpha}$ will denote the αth determination in the ith series.

1. Subtract from every one of these values a same constant, chosen as a round number near a roughly estimated mean of the x-values, and multiply the differences thus obtained by a same factor, say K, chosen in such a way as to make the resulting values small, with at most one decimal place. For example, from 18.22, 18.32, 18.175, 18.26, etc., we obtain first: 0.02, 0.12, −0.025, 0.06, etc., and finally: 2, 12, −2.5, 6, etc. In this case $K = 100$.

Suppose that we thus obtain the values $y_{i\alpha}$, arranged according to the same scheme:

1st series: $y_{11}\ y_{12}\ y_{13} \ldots y_{1N}$
2d series: $y_{21}\ y_{22}\ y_{23} \ldots y_{2N}$
. . . . . .
. . . . . .
Mth series: $y_{M1}\ y_{M2}\ y_{M3} \ldots y_{MN}$

2. Now compute:

a. The sum, T_i, of the elements y in the ith series (row); take the square $(T_i)^2$ of this sum. Do this for all rows (all i) and sum all $(T_i)^2$, thus obtaining: $\sum_{i=1}^{M} (T_i)^2$.

b. The global sum of all y's, denoted by T (obviously $T = \sum_{i=1}^{M} T_i$). Take the square, T^2, of this global sum.

c. The sum of the squares $y_{i\alpha}^2$ of all individual y-values—i.e., $\sum_{i=1}^{M} \sum_{\alpha=1}^{N} y_{i\alpha}^2$.

3. From these three quantities, $\sum_i (T_i)^2$, T^2, and $\sum_i \sum_\alpha y_{i\alpha}^2$, derive the following:

$$A = \left[\sum_i \sum_\alpha y_{i\alpha}^2\right] - \frac{1}{N}\left[\sum_i (T_i)^2\right] \tag{1}$$

$$B = \frac{1}{N}\left[\sum_i (T_i)^2\right] - \frac{1}{MN}\left[T\right]^2 \tag{2}$$

$$D = \frac{A}{B}\,\frac{M-1}{M} \tag{3}$$

4. By entering, for the proper values of M and N, Tables I and II, two values, F_0 and F_0', are found, respectively. Compute:

$$D_1 = \frac{D}{F_0} \tag{4}$$

$$D_2 = DF_0' \tag{5}$$

Table I. Values of F_0[a]

M \ N	2	3	4	5	6	7	8	9	10
4	28.7	27.5	27.0	26.8	26.7	26.6	26.5	26.5	26.4
5	15.5	14.5	14.2	14.0	13.9	13.8	13.8	13.7	13.7
6	10.7	9.9	9.6	9.5	9.4	9.3	9.3	9.3	9.2
8	6.8	6.3	6.1	6.0	5.9	5.9	5.8	5.8	5.8
10	5.3	4.8	4.6	4.6	4.5	4.5	4.5	4.4	4.4
12	4.4	4.0	3.9	3.8	3.8	3.7	3.7	3.7	3.7
15	3.7	3.3	3.2	3.2	3.1	3.1	3.1	3.1	3.1
20	3.0	2.8	2.7	2.6	2.6	2.6	2.6	2.6	2.6
30	2.4	2.2	2.2	2.1	2.1	2.1	2.1	2.1	2.1

[a] 1% critical point of F for $M(N-1)$ and $M-1$ degrees of freedom.

Table II. Values of F_0'[a]

M \ N	2	3	4	5	6	7	8	9	10
4	16.7	7.6	6.0	5.3	4.9	4.7	4.6	4.5	4.4
5	11.4	6.0	4.9	4.4	4.2	4.0	3.9	3.8	3.8
6	8.8	5.1	4.3	3.9	3.7	3.6	3.5	3.4	3.4
8	6.2	4.0	3.5	3.3	3.1	3.0	3.0	2.9	2.9
10	5.0	3.5	3.1	2.9	2.8	2.7	2.7	2.6	2.6
12	4.2	3.1	2.8	2.6	2.6	2.5	2.5	2.4	2.4
15	3.6	2.7	2.5	2.4	2.3	2.3	2.3	2.2	2.2
20	3.0	2.4	2.2	2.1	2.1	2.0	2.0	2.0	2.0
30	2.4	2.0	2.0	1.9	1.8	1.8	1.8	1.8	1.8

[a] 1% critical point of F for $M-1$ and $M(N-1)$ degrees of freedom.

5. From D, D_1, and D_2 we now obtain:

a.
$$Est\ [CI]_N = 1 + D \tag{6}$$

and the corresponding confidence limits [if $L_2 > N$, take $L_2 = N$ (cf. section IV)]:

$$L_1 = 1 + D_1 \tag{6a}$$

$$L_2 = 1 + D_2 \tag{6b}$$

b.
$$Est\ [CI]_\infty = \frac{(N-1)(1+D)}{N-(1+D)} \tag{7}$$

and the corresponding confidence limits:

$$L_1' = \frac{(N-1)L_1}{N-L_1} \tag{7a}$$

$$L_2' = \frac{(N-1)L_2}{N-L_2} \tag{7b}$$

c. For any n and n' one can also calculate:

$$Est\ [CI]_n^{n'} = \frac{1}{R}\,\frac{N-1}{N}\,\frac{1+D}{D} \tag{8}$$

where

$$R = \frac{1}{n} + \frac{1}{n'c^2} \tag{8c}$$

[c is the ratio of the orders of magnitude of the absolute errors in the sample determination and in the blank. In general, $c = 1$ (cf. Section II)]

and the corresponding confidence limits:

$$L_1'' = \frac{1}{R}\,\frac{N-1}{N}\,\frac{L_2}{L_2-1} \tag{8a}$$

$$L_2'' = \frac{1}{R}\,\frac{N-1}{N}\,\frac{L_1}{L_1-1} \tag{8b}$$

IV. INTERPRETATION FROM THE RESEARCH ANALYST'S POINT OF VIEW

Consider the scheme of analyses of a sample as shown in the previous section, but suppose that the number of rows is considerably extended—i.e., that a very great number of series of N replicates is available. Now suppose that we compare the two following values:

1. The mean value for a given row (N simultaneous determinations), this row being chosen at random.
2. The mean value obtained by choosing N rows at random and selecting at random one value in each of these rows; then taking the average of these N numbers.

Both these means result from N observations; which is the better from the precision point of view? It is easily seen that on account of the bias which may affect all values of a same row, we have a better chance of obtaining a precise mean value by taking the second average than by taking the first. We are therefore justified in expecting, for a same number, N, of determinations, a higher average precision in the second case than in the first; or to obtain the same precision by using, in the second case, a number of determinations N' smaller than N. The coefficient of improvement $[CI]_N$ is precisely this number, N'. Thus from the precision point of view, the mean value of N determinations made in parallel—the usual case in practice—is equivalent to only N' ($\leqslant N$) truly random determinations. As pointed out previously, $[CI]_N$—i.e., N'—is a "population parameter". Formula 6 gives but an estimate of this quantity, subject to sampling fluctuations. Here the confidence limits, L_1 and L_2, will prove of value: the confidence coefficient is 0.98, which means that the probability is 0.98 that the interval L_1L_2 covers the true value $[CI]_N$ (cf. *2*).

It is also obvious that the range of possible values for $[CI]_N$ extends from 1 to N. If, therefore, as may well happen, the interval L_1L_2 extends beyond this range, the outside values must be discarded.

Finally, the value 0.98 given for the confidence coefficient must not be taken in too absolute a sense; indeed, any departure from the ideal conditions as outlined in Section I will affect the value of this probability.

Similar considerations apply to $[CI]_\infty$ and $[CI]_n^{n'}$. Whereas for $[CI]_\infty$ a finite upper confidence limit will in general be obtained (unless $L_2 = N$), the value for $[CI]_n^{n'}$ can, of course, become arbitrarily great for n and n' sufficiently large. The reason for this is apparent: $[CI]_n^{n'}$ is the ratio of the precision (inverse of the variance) of a value corrected for bias to that of a single determination subject to a systematic error; by increasing n and n' the precision of the corrected value necessarily approaches the ideal—i.e., ∞—since the random errors approach 0 and no systematic error vitiates the result. The interest of Formulas 7 and 8 lies in the comparison of their numerical values for small values of n and n' (Section VI).

V. ROUTINE ANALYST'S POINT OF VIEW

Let us suppose that an analytical procedure has been investigated by the method described in this paper. A routine analyst now uses the same analytical procedure for N replicate determinations on an unknown sample, and obtains an average value (arithmetic mean of his N values) which we will denote by Z.

The question confronting him is to determine an interval in which it is reasonable to assume that the true value for his sample lies, allowing for all the imperfections which a study of precision has revealed.

Formulas 9 and 10 give, respectively, the lower limit Z_1 and the upper limit Z_2 for such an interval.

$$Z_1 = Z - \frac{t_{(M)}}{K}\sqrt{\frac{B}{N(M-1)}} \tag{9}$$

$$Z_2 = Z + \frac{t_{(M)}}{K}\sqrt{\frac{B}{N(M-1)}} \qquad (10)$$

where B is given by Formula 2, while K is the factor defined in Section III. For $t_{(M)}$ the first or the second value found in Table III for the proper value of M is taken, according as one wishes a 99% or a 95% confidence coefficient—i.e., according as the probability be 0.99 or 0.95 that the interval Z_1Z_2 covers the true value. The latter statement is true only if the analytical method considered is not affected by a systematic error. If such an error should be present and its value known, this value is subtracted from both Z_1 and Z_2 in order to give a correct interval.

The application of Formulas 9 and 10 requires that the number of parallel determinations made by the routine analyst be precisely the number N of replicates in each of the M series of determinations which were made the basis for the statistical study of the analytical method. If the routine analyst made a number N_0 of replicate determinations ($N_0 \neq N$), he cannot use these formulas; in this case a fair approximation for a 99 or 95% confidence interval may be obtained by applying Formulas 11 and 12, provided that the number $M(N-1)$ be fairly large, say not less than 30. Since this number is entirely the choice of the research analyst, it is reasonable to recommend that in the application of the method proposed in this paper, the experiment be arranged so as to make $M(N-1) \geqslant 30$.

On the other hand it can be seen from Table III that for a value of $M < 5$, even the 95% value of $t_{(M)}$ is fairly large, with the result that the corresponding confidence interval is excessively long. In Table IV are offered a few suggestions for the choice of M and N; evidently a higher precision is obtained by increasing these numbers. More advantage is gained by increasing M than by taking N large.

The formulas referred to above are:

$$Z_1' = Z - \frac{t_{(M)}}{K}\sqrt{V'} \qquad (11)$$

$$Z_2' = Z + \frac{t_{(M)}}{K}\sqrt{V'} \qquad (12)$$

where V' is given by:

$$V' = \frac{B}{N(M-1)}\left\{1 + \frac{D}{N-1}\left(\frac{N}{N_0} - 1\right)\right\} \qquad (13)$$

If, on the other hand, the routine analyst had made n_0 blanks in addition to his N_0 sample determinations, and if Z now represents the difference between average "sample" and average "blank", then Formulas 14 and 15 are correct, even for small values of $M(N-1)$:

$$Z_1'' = Z - \frac{t_{(M_0)}}{K}\sqrt{V''} \qquad (14)$$

$$Z_2'' = Z + \frac{t_{(M_0)}}{K}\sqrt{V''} \qquad (15)$$

where V'' is given by:

$$V'' = \left(\frac{1}{N_0} + \frac{1}{n_0c^2}\right)\frac{A}{M(N-1)} \qquad (16)$$

and

$$M_0 = 1 + M(N-1) \qquad (16a)$$

(If this value of M_0 is not found in Table III, a sufficient approximation will be obtained by interpolation.)

Here again, for a fixed value of $N_0 + n_0$, the smallest value of V'', and consequently the smallest confidence interval and highest precision, are obtained when $N_0 = n_0c$.

Table III. Values of $t_{(M)}$[a]

M	4	5	6	8	10	12	15	20	30	50	>50
$t_{(M)}$ (99%)	5.84	4.60	4.03	3.50	3.25	3.11	2.98	2.86	2.76	2.68	2.58
$t_{(M)}$ (95%)	3.18	2.78	2.57	2.36	2.26	2.20	2.14	2.09	2.04	2.01	1.96

[a] For use with Formulas 14 and 15, replace M in this table by M_0, where M_0 is given by 16a. Interpolate if necessary.

Table IV. Choice of M and N

M	5	6	8	10	12	15	20	30	>30
N	7	6	5	4	4	3	3	2	2

VI. NUMERICAL ILLUSTRATION

In Table VI the simple calculations described in Section III are applied to Moran's data, reproduced in Table V. Not more than 15 to 20 minutes of simple arithmetic, using an ordinary slide rule and perhaps a table of squares, are required. Considering the time usually devoted to the purely experimental work, it is therefore not unreasonable to recommend those few calculations, which lead to a much more objective appreciation of precision than can be gained from mere visual inspection or even by graphical methods.

In order to show this more clearly, the method was also applied to another example found in the recent literature (*9*).

Williams and Haines, in presenting a new determination of sodium in potassium hydroxide, studied the precision of their method under routine conditions by making duplicate deter-

Table V. Precision of Determination of Specific Gravity of CCl_4 under Routine Conditions

Jan.	1.5850	1.5851	July	1.5849	1.5848
Feb.	1.5848	1.5850	Aug.	1.5854	1.5854
March	1.5846	1.5846	Sept.	1.5852	1.5853
April	1.5851	1.5851	Oct.	1.5847	1.5851
May	1.5853	1.5853	Nov.	1.5850	1.5850
June	1.5849	1.5850	Dec.	1.5849	1.5851

Table VI. Estimation of $[CI]_2$, $[CI]_\infty$, and $[CI]_n^{n'}$ for Specific Gravity Determination of CCl_4

Subtracting 1.5850 from all values and taking $K = 10{,}000$

Month	y_{i1}	y_{i2}	T_i	T_i^2	y_{i1}^2	y_{i2}^2	$y_{i1}^2 + y_{i2}^2$
Jan.	0	1	1	1	0	1	1
Feb.	−2	0	−2	4	4	0	4
Mar.	−4	−4	−8	64	16	16	32
Apr.	1	1	2	4	1	1	2
May	3	3	6	36	9	9	18
June	−1	0	−1	1	1	0	1
July	−1	−2	−3	9	1	4	5
Aug.	4	4	8	64	16	16	32
Sept.	2	3	5	25	4	9	13
Oct.	−3	1	−2	4	9	1	10
Nov.	0	0	0	0	0	0	0
Dec.	−1	1	0	0	1	1	2
	$T = 6$						
	$T^2 = 36$		$\sum_i T_i^2 = 212$				$\sum_i\sum_\alpha y_{i\alpha}^2 = 120$

$M = 12$; $N = 2$; hence (cf. Tables I and II): $F_0 = 4.4$ and $F_0' = 4.2$

$A = 120 - (212/2) = 14 \qquad B = (212/2) - (36/24) = 104.5$

Hence: $D = (14/104.5) \times (11/12) = 0.123$

$D_1 = 0.123/4.4 = 0.03 \qquad D_2 = (0.123) \times (4.2) = 0.52$

Thus: $Est[CI]_2 = 1 + 0.12 = 1.12$

$L_1 = 1 + 0.03 = 1.03 \qquad L_2 = 1 + 0.52 = 1.52$

$$Est\,[CI]_\infty = \frac{1 + 0.123}{2 - (1 + 0.123)} = 1.27$$

$L_1' = 1.03/(2 - 1.03) = 1.06 \qquad L_2' = 1.52/(2 - 1.52) = 3.16$

Assuming $c = 1$, we further obtain:

$$Est\,[CI]_1^1 = \frac{1}{2}\frac{1}{2}\frac{1.123}{0.123} = 2.28$$

$$L_1'' = \frac{1}{2}\frac{1}{2}\frac{1.52}{0.52} = 0.73 \qquad L_2'' = \frac{1}{2}\frac{1}{2}\frac{1.03}{0.03} = 8.6$$

and likewise:

$Est\,[CI]_2^2 = 4.56$ with the confidence limits 1.46 and 17.3.

minations every month over a period of 20 months. The application of the author's formulas to these data (omitting the second of the three determinations made in January, 1942) gives: $M = 20$; $N = 2$; for $K = 1000$, we find $B = 613.25$; $D = 0.786$; $D_1 = 0.26$; $D_2 = 2.32$. Hence: $Est[CI]_2 = 1.79$; $L_1 = 1.26$; $L_2 = 3.32$.

Since of necessity $L_2 \leqslant 2$ (cf. Section IV), we take $L_2 = 2$.

We further find: $Est[CI]_\infty = 8.5$; $L_1' = 1.70$ and $L_2' = \infty$; and—assuming that a blank is made with the same (absolute) precision as a "sample"—$Est[CI]_1' = 0.57$; $L_1'' = 0.50$ and $L_2'' = 1.21$.

A rather striking difference appears when these values are compared with the results of Table VI: there little advantage was gained by infinite replication (a result consistent with the low $[CI]_2$), while only two blanks run in parallel with duplicate determinations resulted in a very definite improvement in precision. (Obviously, infinite replication is a purely theoretical concept. From a practical point of view it is to be considered as the best that can be obtained by running a very great number of replicates under nearly constant conditions.) In the case of the sodium determination, on the contrary, $[CI]_2$ is rather high: replication therefore improves the precision considerably. The running of a blank in the latter case is justified only inasmuch as it improves the accuracy of the method—i.e., inasmuch as it eliminates a constant systematic error inherent in the method—but in contradistinction to the case exemplified in Table VI, a blank made once for all is then perfectly satisfactory.

VII. UNDERLYING THEORY

Referring to the scheme of x-values in Section III, we find for the estimate of the variance σ^2 "within" rows (*4*, *5*, *6*):

$$\sum_{i=1}^{M}\sum_{\alpha=1}^{N}(x_{i\alpha} - x_i)^2/M(N-1)$$

where x_i is the mean of the ith row. The variability of x_i itself is composed of two parts: the variance σ'^2 of the "true" means of rows and, in addition, the variability resulting from its being a sample mean of N values of variance σ^2—i.e., σ^2/N. Thus the expression $\sum_{i=1}^{M}(x_i - \bar{x})^2/(M-1)$, in which $\bar{x}$ is the grand average of the x-values, is an estimate for $\sigma'^2 + \sigma^2/N$.

From Formulas 1 and 2 it can easily be seen that the two statistics just mentioned are equal, respectively, to $\left(\frac{A}{K^2}\right)\Big/ M(N-1)$ and $\left(\frac{B}{NK^2}\right)\Big/(M-1)$. These two estimates being statistically independent (*2*, *4*, *8*), the ratio

$$\left[\frac{A/K^2M(N-1)}{\sigma^2}\right]\Big/\left[\frac{B/NK^2(M-1)}{\sigma'^2 + \sigma^2/N}\right]$$

has the F distribution (*2*, *4*, *5*, *6*) with $M(N-1)$ and $(M-1)$ degrees of freedom. [An exact formulation of the theorem involved in this statement will be found in Example 1, on page 115, of (*8*).] Hence:

$$\left(\frac{\sigma'^2}{\sigma^2} + \frac{1}{N}\right)\frac{A}{B}\frac{N(M-1)}{M(N-1)} = F$$

Let $\frac{\sigma'^2}{\sigma^2} = \varphi^2$; using Formula 3 we obtain:

$$F = \left(\varphi^2 + \frac{1}{N}\right)D\,\frac{N}{N-1} \tag{17}$$

Now, by definition

$$[CI]_N = \frac{\sigma'^2 + \sigma^2}{\sigma'^2 + \sigma^2/N} = \frac{\varphi^2 + 1}{\varphi^2 + \frac{1}{N}} \tag{18}$$

$$[CI]_\infty = \frac{\sigma'^2 + \sigma^2}{\sigma'^2 + \frac{\sigma^2}{\infty}} = \frac{\sigma'^2 + \sigma^2}{\sigma'^2} = 1 + \frac{1}{\varphi^2} \tag{19}$$

$$[CI]_n^{n'} = \frac{\sigma'^2 + \sigma^2}{\sigma^2\left[\frac{1}{n} + \frac{1}{n'c^2}\right]} = \frac{1}{\frac{1}{n} + \frac{1}{n'c^2}}(\varphi^2 + 1) = \frac{1}{R}(\varphi^2 + 1) \tag{20}$$

Solving 18 for φ^2, we obtain:

$$\varphi^2 = \frac{1 - [CI]_N/N}{[CI]_N - 1}$$

Hence:

$$\varphi^2 + \frac{1}{N} = \frac{N-1}{N}\,\frac{1}{[CI]_N - 1} \tag{21}$$

Substitution of 21 in 17 yields:

$$F = \frac{D}{[CI]_N - 1} \tag{22}$$

Now F_0 is the 1% tabular value of the F-statistic (*4*, *5*, *6*) for $M(N-1)$ and $(M-1)$ degrees of freedom, while F_0' is the 1% tabular value for $(M-1)$ and $M(N-1)$ degrees of freedom. Therefore lower and upper limits for $[CI]_N$, for a 98% confidence coefficient (*2*, *4*, *8*), are obtained by solving for L_1 and L_2 the equations

$$F_0 = \frac{D}{L_1 - 1} \quad \text{and} \quad \frac{1}{F_0'} = \frac{D}{L_2 - 1}$$

which yield immediately Formulas 6a and 6b, by means of 4 and 5.

A point-estimate (*1*, *4*, *7*, *8*) for $[CI]_N$ is obtained by making $F = 1$ in Formula 22; hence 6.

The groups of Formulas 7 and 8 are similarly obtained by solving, respectively, Equations 19 and 20 for φ^2 and substituting in 17.

The formulas of Section V are immediate applications of "Student's t distribution" (*1*, *2*, *4*, *8*): $t_{(M)}$ is the tabular value for $M-1$ degrees of freedom on either the 1% or the 5% level of significance (*2*, *4*, *6*, *8*). In Formulas 9 and 10 the estimate of the variance is $B/K^2N(M-1)$, corresponding to $\sigma'^2 + \frac{\sigma^2}{N}$.

Formula 13 provides an estimate of the variance $\sigma'^2 + \frac{\sigma^2}{N_0}$, but assumes that σ^2 is exactly equal to $A/K^2M(N-1)$. [It is for this reason that we assumed, in this case, the number of degrees of freedom $M(N-1)$ sufficiently great.] Formula 13 then easily follows from the identity $\sigma'^2 + \frac{\sigma^2}{N_0} = \left(\sigma'^2 + \frac{\sigma^2}{N}\right) + \sigma^2\left(\frac{1}{N_0} - \frac{1}{N}\right)$, remembering that the estimate of $\sigma'^2 + \frac{\sigma^2}{N}$ is $B/K^2N(M-1)$ and introducing 3.

Finally, Formulas 14 and 15 necessitate the variance of $\bar{x}_{N_0} - \bar{b}_{n_0}$ ($\bar{x}_{N_0}$ is the average of N_0 determinations of x, while $\bar{b}_{n_0}$ is the average of n_0 "blanks"), which is $\sigma^2\left(\frac{1}{N_0} + \frac{1}{n_0c^2}\right)$, the estimate of which is V'', as given by 16. Here the number of degrees of freedom is $M(N-1)$; thus $M_0 - 1 = M(N-1)$; hence 16a

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Determining Rates of Deoxidation of Iron Oxide Materials in Reducing Gases

Loss-in-Weight Method

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In determining the rates of deoxidation of iron oxide materials in reducing gases, iron oxide material is heated in an alloy container suspended from a balance, and reducing gas at a controlled rate of flow is passed through the heated material. The loss in weight (oxygen) is recorded at intervals until almost constant weight is obtained. Method is simple, accurate, and rapid.

THE successful use of fluidized solid catalysts for increasing reaction rates in some industrial processes led to the belief that this principle could be used advantageously in the deoxidation of fine iron ores. Fluidization used in this sense means suspension of the finely ground ore in a gas stream of suitable velocity to cause the mixture to behave more or less like a true fluid. The apparatus described in this report was designed and built for studying various factors governing the deoxidation of iron ores fluidized in hydrogen at 575° to 600° C. The results were so satisfactory that the apparatus was modified slightly for use in determining the relative rates of deoxidation of iron oxide materials by direct weighing at any time interval.

LOSS-IN-WEIGHT APPARATUS

The apparatus consists of a reaction tube of Pyrex or heat-resisting alloy 1.7 inches in diameter by 12 inches long, with the lower end closed. Hydrogen is introduced through a heat-resisting alloy tube and distributor extending to the bottom of the reaction tube. A 2-inch layer of −10- +14-mesh mullite grog placed above the metal distributor serves as a heater for the hydrogen and as an additional distributor for the hot hydrogen.

The reaction tube is supported in a stainless steel basket fastened to a disk on the hydrogen inlet tube. An alloy rod serves as a suspension link between the hydrogen inlet tube and a stirrup over one pan of a torsion balance. Unused hydrogen and water vapor from the reaction pass directly into the furnace atmosphere. A plate-type water cooler between the balance and the heater prevents the balance from becoming hot. A schematic drawing of the apparatus is shown in Figure 1 and details are shown in Figure 2.

The maximum temperature at which a Pyrex reaction tube can be used is 700° C.; at higher temperatures it is necessary to use a stainless steel retort. Other parts of the apparatus are unchanged. The rate of deoxidation of iron oxide material not larger than 0.525 inch may be determined at temperatures not exceeding 1000° C. A larger unit, embodying the same principles, has been constructed for determining the rates of deoxidation of larger sizes of ore in water gas.

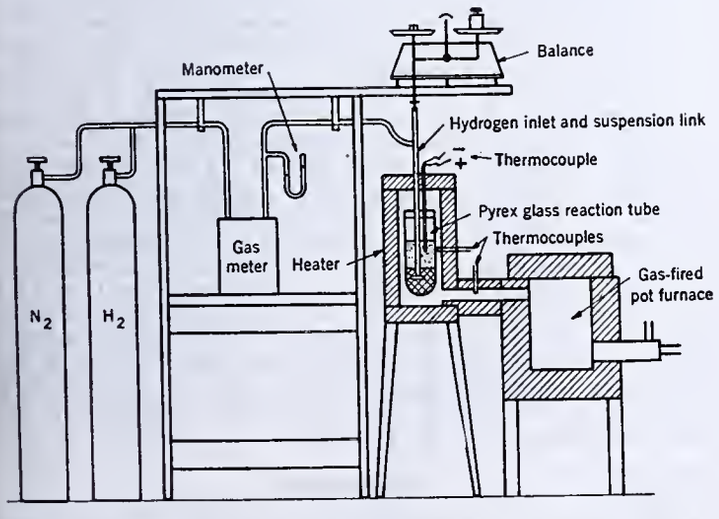

Figure 1. Schematic Drawing of Apparatus for Studying Deoxidation of Iron Ores in Hydrogen

PREPARATION OF SAMPLE FOR DEOXIDATION

Results of numerous tests have shown that the following procedure is suitable for preparing samples of iron ores:

A 100-pound sample of ore is screened on 0.525-inch. The oversize material is crushed to pass 0.525-inch and is added to the original undersize product. The combined −0.525-inch product is wet-screened on 20-mesh; the +20-mesh material is dried at 105° C. and separated into four sizes as follows: −0.525-inch +3-mesh; −3- +6-mesh; −6- +10-mesh; and −10- +20-mesh. A 227-gram (0.5-pound) composite is made of the four sizes and used for the deoxidation test. A duplicate composite is ground to −100-mesh for chemical analysis.

TEST PROCEDURE

The retort assembly containing the oxide sample is suspended from the balance, and the insulating brick top of the heater is replaced. The cover is removed from the pot furnace, and the gas burner is started. When the lining is red-hot the cover is replaced, forcing the hot products of combustion into the heater surrounding the retort. Nitrogen is passed through the retort to facilitate heating the ore charge, to sweep out any volatile components in the ore, and to provide an inert atmosphere. When the desired temperature is attained in the charge and the weight of the charge is constant, the nitrogen flow is stopped and the hydrogen flow is started. A good deoxidation rate is obtained with a hydrogen flow of about 0.25 cubic feet a minute. The loss in weight is recorded at regular intervals, usually every 5 minutes, until constant weight is attained or the deoxidation has proceeded as far as desired. After the gas burner has been shut off, the hydrogen flow is continued until the retort has reached room temperature. Cooling may be expedited by removing the retort from the furnace. When cool, the product is removed from the retort.

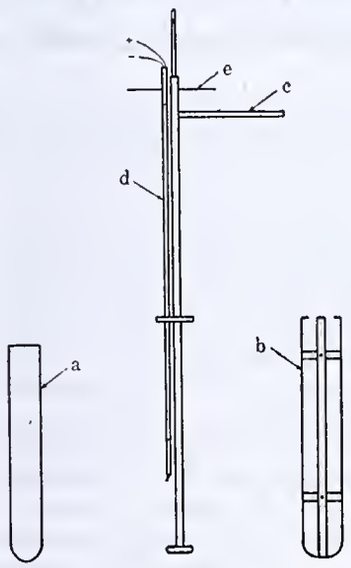

Figure 2. Parts of Loss-in-Weight Apparatus

a. Reaction tube
b. Stainless steel basket
c. Hydrogen inlet and connection to balance
d. Thermocouple
e. Heat deflector

The deoxidation is computed for each period by dividing the weight of oxygen lost during that period by the total available oxygen in the charge. Available oxygen in the charge is computed from the iron and manganese assays of the composite. The total loss of oxygen at constant weight will agree closely with the computed available oxygen if the test has been made properly. The loss in weight of the charge in nitrogen represents the ignition loss at the temperature of the test.

COMPARISON WITH OTHER METHODS

The laboratory method commonly used by the Bureau of Mines at its Minneapolis station for determining the rate of deoxidation

Table I. Log of a Typical Deoxidation Test

Weight of ore	227 grams
Size of ore	−0.525-inch +20-mesh
Temperature of test	900° C.
Rate of hydrogen flow	0.23 cu. ft. per minute
Available oxygen in sample	59.0 grams

Time after Start of H_2	Temperature In heater	Temperature In ore bed	Pressure Drop through Ore Bed	Gross Weight	Deoxidation Cumulative	Hydrogen Utilization
Min.	*° C.*	*° C.*	*Cm. water*	*Grams*	*%*	*%/time interval*
..	25	25	0.0	1,519.3	0.0	0.0
..	890	868	3.3	1,515.4	0.0	0.0
..	916	890	3.3	1,515.4	0.0	0.0
0	916	904	3.4	1,515.4	0.0	0.0
10	912	852	5.8	1,488.0	46.4	63.9
15	912	855	5.6	1,480.0	60.0	37.3
20	920	880	5.5	1,473.0	71.8	32.6
25	920	892	5.4	1,467.2	81.7	27.0
30	920	894	5.3	1,463.2	88.4	18.6
35	920	894	5.3	1,460.3	93.4	13.5
40	916	890	5.2	1,458.6	96.2	7.9
45	912	890	5.1	1,457.2	96.9	2.8
50	914	890	5.1	1,456.5	98.1	2.3
55	916	890	5.1	1,456.3	98.4	0.93
60	918	892	5.1	1,456.2	98.6	0.47
					Over-all H_2 utilization	22.6

of iron oxide materials has been to heat 1 to 20 grams of the oxide in a tube furnace while hydrogen was passed over the heated material. Water formed by the reaction was absorbed and weighed at regular intervals. The apparatus and procedure for determining rates of deoxidation are described in a technical press article (*2*).

After comparing the porosity of a group of iron ores with their respective reducibility, the data (*1*) obtained by the Bureau of Mines indicated that the porosity of an ore could be used as an index of its relative reducibility and of the size to which it should be crushed for the most efficient use in the blast furnace.

The loss-in-weight method has the following advantages:

The larger weight of material used permits more accurate sampling.

The larger weight of oxygen removed minimizes errors in absorbing and weighing small quantities.

Absorption of products of reaction is eliminated.

Errors due to part of the water being condensed in the reaction tube and not absorbed during the period in which it is formed are avoided.

Only one test is necessary to determine the course of deoxidation over its entire range.

Sampling and analyzing the deoxidized products are eliminated.

Rates of deoxidation in hydrogen, in carbon monoxide, or in mixtures of hydrogen and carbon monoxide may be determined.

Utilization of the reducing gas may be determined for each time interval from the beginning to the end of the test and at any degree of deoxidation.

The method is simple, rapid, and accurate.

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Estimation of Naphthalene in Absorbing Oil

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IN CONNECTION with a project at this laboratory involving pilot-plant studies of a process for stripping naphthalene from spent scrubbing oil, a rapid method for the estimation of naphthalene in oil was needed.

The method in general usage in the manufactured gas industry employs volatilization of the naphthalene by means of a stream of hot air, and subsequent precipitation of the naphthalene in a solution of picric acid (*2*). This procedure requires from 15 to 20 hours to perform and is cumbersome to control. Another method requires the distillation of the spent oil, and isolation of the fraction boiling between 195° and 250° C. This fraction is then chilled and the solid naphthalene is filtered and weighed (*3*). This procedure is also rather lengthy and affords considerable opportunity for inaccuracies. Bliss (*4*) reports a control test involving the refractive index of the oil. This method, developed from the work in oil evaluation by Kugel and Bliss (*5*), would have required considerable background study which at the time was not possible. The method of White (*6*), which involves bromination of the oil, solution of the brominated oil in acetone, and subsequent precipitation of naphthalene as the picrate, was not considered satisfactory for the purpose, since it involves considerable manipulation throughout the analysis.

Preliminary attempts were made to effect a separation of naphthalene from the oil by solvent extraction. Tests made by extraction with acetic acid and by shaking the spent oil with saturated picric acid were unsuccessful. It was therefore decided to concentrate on a modification of the volatilization procedure described above, which would effect the separation of naphthalene from a reasonably sized sample of oil in as short a time as possible and with a minimum of analytical manipulation.

REAGENTS

Sodium hydroxide, 0.1 *N*.
Methyl red indicator, 0.1% in ethanol.

Standard Picric Acid. Forty to 50 grams of the analytical reagent are added to approximately 2 liters of distilled water and the mixture is heated to 150° F. The mixture is then cooled to 64° to 66° F. and is filtered. The filtrate is diluted with distilled water until a 100-ml. portion is equivalent to 48 to 50 ml. of 0.1 *N* sodium hydroxide.

Naphthalene-free absorbing oil.

APPARATUS

Figure 1 is a line drawing of the apparatus finally evolved for the test. The apparatus consists essentially of a partly packed glass stripping tower jacketed for circulating hot water, a constant-temperature system for natural circulation of water, a sample container, and a four-bottle absorption train. The sample tube, *A*, may be disconnected from the apparatus and the oil sample weighed directly therein. The stripping tower, *B*, is partly packed with 6-mm. glass beads. The column of packing is 18 cm. high and is supported by projections in the base of the tube. The bulb at the top of *B* is designed to lower the velocity of the exit gases and reduce oil entrainment. It also serves to reduce the exit air temperature to approximately room temperature. The proper tube temperature is maintained by the circulation of hot water. The heating spiral, *C*, is B. & S. No. 22 Chromel wire and is connected in series with a resistor (not shown in Figure 1) and a bimetallic thermoregulator, *D*, rated at 10 amperes at 115 volts alternating current. Under the test conditions, the temperature of the circulating water may be maintained within ±5° F. of that required, without using forced circulation. The stopcock, *G*, is used as a drain for the circulating system.

Dry air is supplied through a constant-pressure regulator (a mercury-column type regulator not shown in Figure 1) and a U-tube flowmeter, *E*, to *A*. The oil contained in tube *A* is thus forced up into the stripping tower, and the air and naphthalene vapors pass out of the tower into the absorption bottles, *F*, each containing 100 ml. of standard picric acid solution. With the exception of the water-jacket connections, best quality corks are used throughout the apparatus.

PROCEDURE

The sample of oil, containing not more than 170 mg. of naphthalene, is weighed directly into the sample tube, and naphtha-

lene-free oil is added, if necessary, to bring the total volume to 10 ± 1 ml. The sample tube is then connected to the base of the stripping tower and the temperature of the circulating water is adjusted to 185° F. Into each of the four absorption bottles are pipetted 100 ml. of standard picric acid solution. The bottles are then connected in series at the outlet of the stripping tower, care being taken to make all connections glass to glass, since naphthalene is appreciably absorbed by rubber tubing. Dry air is then passed through the stripping tower at a rate of 3 cubic feet per hour. At the end of one hour the air rate is increased to 4 cubic feet per hour and is thus maintained for 2 hours. At these rates of air flow, the oil sample is entirely supported in the stripping tower.

The naphthalene is volatilized by the warm air and reacts with the picric acid to form a precipitate of naphthalene picrate, thus reducing the titer of the picric acid. The absorbing solution should be maintained between 65° and 80° F. during the analysis. When the 3-hour stripping period is completed, the contents of the four absorption bottles, including the precipitate, are quantitatively transferred to a 500-ml. volumetric flask and made up to the mark. Only 100 ml. of distilled water are used for washing in order to reduce the solubility of naphthalene picrate to a minimum. It is advisable to use a wide-necked volumetric flask and a powder funnel for the transfer, since the precipitate of naphthalene picrate may be agglomerated because of slight oil carry-over. The contents of the volumetric flask are well mixed and filtered through paper into a clean, dry flask. The first 100 ml. of filtrate are rejected and of the remaining filtrate, 100 ml. are titrated with 0.1 N sodium hydroxide using methyl red as indicator. This titer is then corrected to the basis of original undiluted picric acid. The difference in titer of the 400 ml. of picric acid before and after naphthalene absorption is converted to per cent by weight of naphthalene in the original oil sample. This is essentially the method of Sperr and Powell (*1*).

TEST OF ACCURACY

The method was checked using samples of oil containing known amounts of pure naphthalene. The oil used in making up the samples for the tests listed in Table I was Essodiesel 210, marketed by the Standard Oil Company of New Jersey.

The tests listed in Table I are selected from fifty or more tests made during the investigation. As may be expected, the accuracy decreases with decreasing naphthalene concentrations, largely because of the smaller differences in titer at the lower naphthalene concentrations.

Several of the tests included in Table I were run in 2.5 hours. When the naphthalene content is on the order of 1% or less, the runs may safely be made in this time. It is not recommended that the time be further reduced, since experience with running times of 2 hours or less has produced low results. However, when analyzing oils containing in excess of 2% naphthalene (when the sample for analysis must be diluted with naphthalene-free oil), it is best to take the full 170 mg. of naphthalene for analysis. This allows a relatively high titer difference and gives greater accuracy.

Table I. Estimation of Naphthalene

Time of Stripping	Temperature	Total Air	Naphthalene	
			Actual	Test
Hours	*° F.*	*Cu. feet*	*% by weight*	
3.0	185	11	2.00	2.0
3.0	185	11	2.00	2.0
3.0	185	11	2.00	2.0
3.0	185	11	1.48	1.4
3.0	185	11	1.32	1.3
3.0	185	11	1.32	1.2
3.0	188	11	1.01	1.0
2.5	188	9	1.01	1.0
2.5	185	9	1.01	1.1
2.5	185	9	1.01	1.0
2.5	185	9	0.50	0.4
2.5	185	9	0.50	0.4

DISCUSSION

The matter of determining the optimum temperature at which to conduct the stripping procedure was of considerable importance. The amount of naphthalene removed from an oil in a given time varies with the stripping temperature and the volume of air used. Under the test conditions, the air rate is limited by the time of contact required for complete absorption of the naphthalene in the picric acid. It was found that 4 cubic feet per hour was the highest air rate which could be considered. A number of tests were made to determine the proper stripping temperature; temperatures in excess of 200° F. produced erratic results. This was largely due to the type of oil under test. At the higher temperatures a considerable portion of the oil was distilled over with the naphthalene, causing difficulty in carrying through the volumetric procedure. A distillation test of Essodiesel 210 is given in Table II.

Table II. Distillation, A.S.T.M.

	Essodiesel 210
	° F.
Initial boiling point	419
5% recovered at	...
10%	468
50%	548
90%	640
Maximum temperature	675
Distillation recovery, %	98

Preliminary tests were conducted with other oils used as naphthalene absorbents. These oils distill at temperatures higher than Essodiesel 210 and in a narrower temperature range. Samples containing known amounts of naphthalene were prepared from these oils and check determinations were made on the apparatus. The results were generally satisfactory, although in several instances consistently low values were obtained. This led to the conclusion that, in the case of some oils, a definite percentage of naphthalene is retained in the oil and may not easily be removed by air volatilization. Therefore, when working with oils other than Essodiesel 210, initial runs should be made using samples containing known amounts of naphthalene in order to

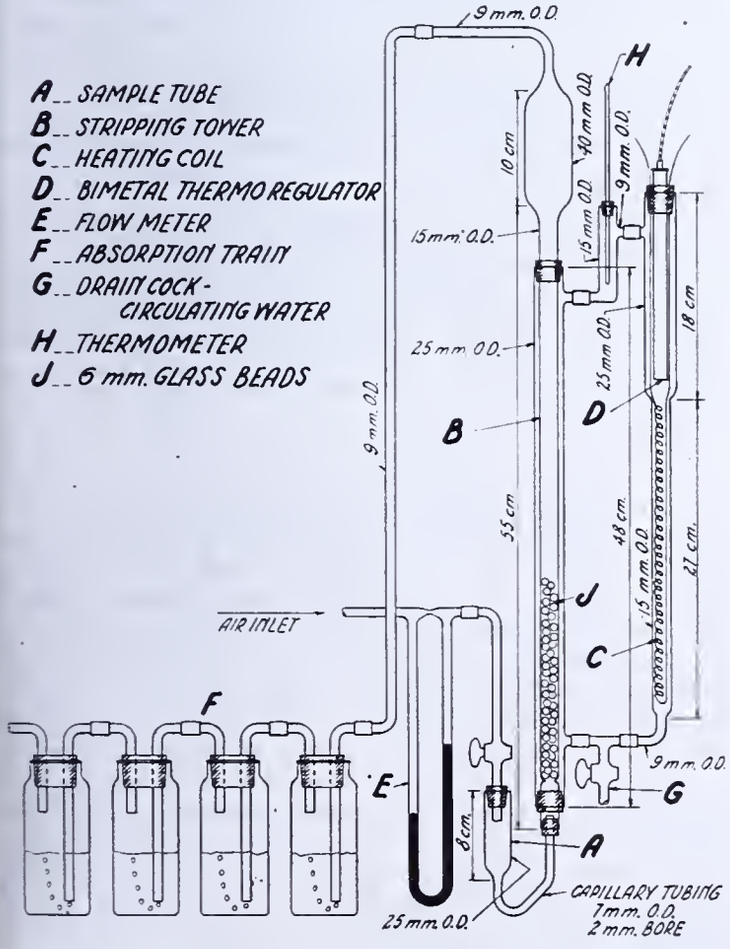

Figure 1. Diagram of Apparatus

determine the extent of recovery possible with air volatilization in the recommended time.

ACKNOWLEDGMENT

The authors wish to thank Ruth Votaw, formerly of this laboratory, for her assistance in the analytical work. They also express appreciation to R. Fram for preparation of the drawing, and to T. L. Robey and J. E. Brewer for helpful suggestions during the course of the investigation. The cooperation of the laboratories of the Consolidated Gas, Electric Light and Power Company of Baltimore, the Eastern Gas and Fuel Associates, the Philadelphia Electric Company, and the Rochester Gas and Electric Corporation in supplying oil samples is gratefully acknowledged.

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Determination of Vitamin A in Liver

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A rapid procedure is described for the colorimetric determination of vitamin A in liver. Vitamin A is quantitatively recovered from saponified 1-gram samples of liver by a single extraction with petroleum ether. Negligible amounts of interfering substances permit direct evaporation of the petroleum ether and treatment of the residue in chloroform with antimony trichloride. Errors due to soap formation, reagent impurities, and light are briefly discussed.

THE vitamin A content of a number of materials can be accurately determined by modified colorimetric methods (*4*, *8*, *9*) based on the reaction of vitamin A with antimony trichloride in chloroform. Procedures for the extraction of vitamin A and removal of interfering substances previous to color development vary with different materials (*1*, *2*, *3*, *7*) and for the most part require considerable time and careful manipulations. Simplification of these procedures without sacrifice of accuracy in routine testing is desirable, and may be accomplished in the analysis of some materials that are relatively high in vitamin A and low in fat. A simplified procedure similar to that developed for blood (*7*) but with the additional step of saponification (*6*) has been applied to liver after first making a study of the sources of error involved. Among the errors observed are some that have received slight mention in vitamin A methods and others that have been the subject of controversial discussion.

REAGENTS

Potassium hydroxide in aldehyde-free alcohol, 5% solution. The aldehyde-free alcohol is prepared from 95% ethyl alcohol according to the procedure described by Woodman (*11*).

Petroleum ether, boiling point 40° to 60° C. Redistilled Skellysolve F is satisfactory.

Chloroform of reagent grade which meets A.C.S. specifications. If of doubtful quality the chloroform should be specially treated (*8*, *9*).

Antimony trichloride reagent, prepared by rapidly weighing about 100 grams of the C.P. salt from a freshly opened bottle and dissolving it in sufficient chloroform to make a 25% solution. The clear solution should be kept in the dark in a glass-stoppered bottle.

Acetic anhydride, C.P.

PROCEDURE

An Evelyn photoelectric colorimeter with a 620-millimicron filter was used in this work. The advantages of this type of instrument have been discussed by Oser and associates (*9*, *10*). The instrument was calibrated with a chloroform solution of vitamin A esters (Distillation Products, Inc., Rochester, N. Y.) (*9*). The same vitamin A preparation dissolved in suitable solvents was used to perform recovery experiments. The following procedure was employed for the determination of vitamin A in sheep livers and with slight modification has been used successfully for the determination of both carotene and vitamin A in cattle livers. Greater precision may be expected by introducing in the colorimetric phase of this method the internal standard procedure described by Oser, Melnick, and Pader (*9*).

One gram of liver and 5 ml. of 5% aldehyde-free alcoholic potassium hydroxide are placed in a 50-ml. heavy-walled centrifuge tube having a constricted neck and digested in a water bath at 75° C. until the tissue is completely disintegrated. Ten or more samples can be digested conveniently at one time if the concentric rings of a shallow water bath are replaced with a sheet-lead top having 3.1-cm. (1.25-inch) holes. The saponified sample is cooled, brought to its original volume with alcohol, and diluted with an equal volume of water. This concentration of alcohol and potassium hydroxide in the presence of no more than 50 mg. of original fat permits practically complete extraction of vitamin A (and carotene) by petroleum ether without appreciable change in volume. If large amounts of vitamin A are present, an aliquot of the digest is made to a volume of 10 ml. with 50% alcohol.

The contents of the tube are vigorously shaken for 2 to 3 minutes with 10 ml. of petroleum ether and finally centrifuged. Five milliliters of the upper layer, or smaller amounts for livers high in vitamin A, are transferred to an Evelyn colorimeter tube containing a glass bead and evaporated to dryness at 45° C. with the aid of suction. Carotenoids, when present, are determined previous to evaporation of this aliquot by accepted procedures (*3*, *7*, *8*, *9*). The evaporation should be complete within 5 to 10 minutes. A stream of nitrogen may be used as an added precaution against oxidation of vitamin A during evaporation. Ten samples are evaporated at one time with an efficient suction line which prevents condensation of petroleum ether around the stoppers in the necks of the tubes. Most water pumps have too small capacity for this purpose.

The residue in the tube is taken up in 1 ml. of chloroform and after the addition of 1 drop of acetic anhydride the color is developed in the usual manner with 9 ml. of the antimony trichloride reagent. Galvanometer readings at the point of temporary stability are taken within 5 seconds after the addition of the reagent. All operations preceding color development are performed in subdued light. "Low actinic" or amber glassware as suggested by Embree (*5*) will afford further protection against the effects of light.

Consistent results have been obtained from day to day when this procedure has been applied to replicate samples of liver of unknown vitamin A content. Likewise, when vitamin A was added to liver in the form of distilled esters, recovery values agreed within 5% of the calculated amount present. Experience with this procedure under varied conditions showed that fat was an interfering substance, its effect in moderate amounts following saponification being to prevent quantitative extraction of vitamin A with petroleum ether. Although the vitamin could be extracted with diethyl ether in the presence of saponified fat, several extractions followed by thorough washing and drying of the extract were necessary. It seemed desirable to avoid these steps wherever possible. To study some of the limitations of this procedure, recovery experiments were performed with vitamin A added to increasing amounts of vegetable oils and the fortified oils saponified with different amounts of potassium hydroxide and alcohol previous to extraction. Control experi-

Table I. Reliability of Procedure for Determination of Vitamin A in Lamb Liver

Liver Sample No.	Sample Weight, Grams	Micrograms of Vitamin A per Gram Added	Total	Recovered
4	1.00	0	...	8.55
	1.00	2.43	10.98	10.45
	1.00	5.10	13.65	13.70
	1.00	7.75	16.30	16.20
	1.00	13.45	22.00	21.90
	1.00	18.15	26.70	26.65
8	1.03	0	10.86	...
	1.05	0	11.80	...
	1.12	0	11.57	...
	1.11	0	9.94	...
	1.07	0	11.52	...
	1.07	0	10.97	...
		Av.	11.11 (±0.28)	...
21[a]	1.00	0	1.56	...
28[a]	1.05	0	4.42	...
23[a]	1.09	0	19.00	...

[a] Livers 21, 28, and 23 were from lambs of corresponding numbers that received graded levels of carotene after 6 months on a carotene-free ration. No. 21 received approximately 2 mg. of carotene daily; No. 28 was given twice this amount; No. 23 was given twice this amount but for a longer period and was allowed to graze green grass for a week previous to slaughter.

ments were carried out at the same time without oil. In other experiments vitamin A was added at different steps in the procedure.

RESULTS AND DISCUSSION

In Table I are presented values for the recovery of increasing amounts of vitamin A added in the form of distilled esters to a homogeneous sample of lamb liver. The amounts of vitamin A added to the liver were determined colorimetrically in control experiments by direct treatment of the esters in chloroform with antimony trichloride reagent. The intensity of color produced by the esters was found to be the same as that of their unsaponifiable extract, as previously demonstrated by Oser, Melnick, and Pader (*9*). Good agreement between the observed and calculated values shown for sample 4 in Table I gives evidence of the reliability of the procedure for the estimation of vitamin A in liver.

The values shown for liver 8 in Table I were obtained by analyses of samples taken from different sections of the same liver. These values, by comparison with those of the well-mixed sample 4, are indicative of sampling error attributable to an uneven distribution of vitamin A in different parts of the liver. Where greater accuracy is desired, thorough mixing of random samples with due precautions against loss of moisture during mixing would be advisable. Liver samples 21, 28, and 23 were from lambs that received increasing amounts of carotene in their rations.

In Table II are presented values showing the effect of increasing amounts of fat on the recovery of vitamin A by the procedure described. Repetition of these experiments on other occasions and with other oils and fats has demonstrated that in the presence of soap equivalent to no more than 0.05 gram of oil, over 95% of added vitamin A can be extracted from 50% alcohol solutions by a single extraction with petroleum ether. That larger amounts of soap interfere markedly with extraction of vitamin A by petroleum ether is shown throughout the results in Table II. Material other than vitamin A removed by petroleum ether did not interfere with color development, as shown by the results in series 2 when vitamin A was added to the petroleum ether extract of unsaponifiable material just previous to evaporation and color development.

In the extraction of vitamin A with petroleum ether the best separation of layers occurred when the soap solution contained about 50% alcohol. Varying the percentage of alcohol made separation difficult and failed to improve the recovery of vitamin A. Likewise, variation in the concentration of potassium hydroxide (series 3) and in the volume of soap solution (series 4) previous to extraction failed to improve vitamin A recoveries. When the soap solution was extracted with three 10-ml. portions of petroleum ether (series 5) recoveries were improved and approximately 97% of added vitamin A was recovered from 0.10 gram of oil. However, only 57% of added vitamin A could be recovered from 1.0 gram of oil by the modification. Triple extraction with diethyl ether as recommended in most procedures (series 6) apparently effected good extraction of vitamin A unrelated to the amount of soap present; the recovery values, which ranged from 91.1 to 94%, are probably related to losses of vitamin A that occur during washing and drying the extracts.

PRECAUTIONS

In the progress of this work the significance of various sources of error was investigated. Errors due to relatively large amounts of fat have been discussed in relation to the solvent employed for extraction of unsaponifiable material. This error can be avoided, if the sample is sufficiently high in vitamin A, by taking an aliquot of the saponified sample for extraction with petroleum ether. Certain other errors are mentioned here because, despite indications to the contrary in some recently proposed procedures, they have a marked effect on the accuracy of results.

Aldehydes. Alcohol used in saponification must be free of aldehydes to prevent the formation of yellow resins which dissolve in petroleum ether and chloroform and produce a blue color with antimony trichloride. Galvanometer readings equivalent to 0.5 microgram of vitamin A have been observed in blank determinations carried out with ordinary alcohol. This amount of spurious vitamin A produces errors of from 10 to 50% in some determinations.

Rubber Stoppers. Petroleum ether dissolves from new, and some old, rubber stoppers material which reacts with antimony trichloride to give a blue-green color. The intensity of the color, unlike that of the vitamin A reaction, increases during the first few minutes.

Peroxides. The best grades of diethyl ether frequently contain sufficient peroxides to destroy vitamin A, particularly during

Table II. Effect of Fat and Certain Modifications in Procedure on Recovery of Vitamin A

Series	Modification of Procedure	Cottonseed Oil	Vitamin A Added	Vitamin A Recovered	Recovery
		Gram	*Micrograms per gram*		*%*
1	None	0.00	4.80	4.81	100.2
		0.01	4.80	4.85	101.0
		0.02	4.80	4.77	99.3
		0.05	4.80	4.53	94.4
		0.10	4.80	4.04	84.2
		0.20	4.80	3.73	77.7
		0.40	4.80	2.70	56.2
		1.00	4.80	1.56	32.5
2[a]	Vitamin A added to unsaponifiable extract	0.20	3.62	3.66	101.1
		0.40	3.62	3.74	103.3
		0.60	3.62	3.70	102.2
		1.00	3.62	3.70	102.2
3[b]	Concentration of KOH varied	0.20	3.60	2.56	71.1
		0.20	3.60	2.49	69.2
		0.20	3.60	2.70	75.0
		0.20	3.60	2.63	73.1
4[c]	Volume of alcohol-soap-water solution increased	0.10	3.51	3.06	87.2
		0.20	3.51	2.63	74.9
		0.40	3.51	2.22	63.2
5[d]	Triple extraction with petroleum ether	0.00	15.70	15.35	97.8
		0.10	15.70	15.20	96.8
		0.40	15.70	13.35	85.0
		1.00	15.70	8.90	56.7
6[e]	Triple extraction with diethyl ether	0.00	15.70	14.30	91.1
		0.10	15.70	14.75	94.0
		0.40	15.70	14.50	92.3
		1.00	15.70	14.75	94.0

[a] Vitamin A was withheld from saponification mixture and added to petroleum ether extract of unsaponifiable material previous to evaporation.
[b] Concentration of potassium hydroxide in alcohol used for saponification of samples in order given was 2.5, 5.0, 10.0, and 20.0%, respectively. Saponified samples were diluted with an equal volume of water previous to extraction.
[c] Volume of saponified fat solution in 50% alcohol was increased to 30 ml. before extraction with petroleum ether.
[d] Saponified fat solution was extracted three times with petroleum ether. Combined extracts were washed and dried and 1/5th aliquot evaporated.
[e] Same as [d] except that diethyl ether was used for extraction.

separatory funnel operations. When an ether solution containing 2.5 micrograms of vitamin A was evaporated in the usual manner in colorimeter tubes in the presence of 0, 2, 4, 6, and 8 ml. of anhydrous ether which gave a positive peroxide test, losses of vitamin A were 0, 6, 18, 27, and 29%, respectively.

LIGHT. Vitamin A solutions are unstable in bright indoor light (*5*). During a 2-hour period, losses of vitamin A were approximately 38% greater when saponified samples were extracted with ether and the extract washed and dried near a north window than when the same operations were performed in subdued light. Losses of vitamin A in ether solutions placed 180 cm. (6 feet) from a north window, in subdued light, and in the dark over a period of 5 hours were 60, 1.2, and 0%, respectively. Losses in petroleum ether solution under similar conditions were 41, 3, and 0%, respectively.

SUMMARY AND CONCLUSIONS

The vitamin A content of liver can be determined by a single extraction of the saponified sample with petroleum ether, followed by treatment of the evaporated extract with antimony trichloride reagent in the usual manner. The procedure eliminates several manipulations and avoids undue exposure of vitamin A solutions to light. Large amounts of soap in alcohol-water mixtures interfere with the extraction of vitamin A by petroleum ether. Approximately 95% of the vitamin can be recovered, however, when the quantity of soap in the alcohol-water phase does not exceed that equivalent to 0.05 gram of fat.

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Determination of Uronic Acids

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A modified method for determining uronic acids has been developed. Theoretical quantities of carbon dioxide were obtained for all uronic acids and their derivatives by heating the sample with 19% hydrochloric acid in an oil bath maintained at 145° C. for 1.5 to 2 hours. The apparatus is simple, compact, easy to construct, and offers a substantial saving of space and time. Analytical results on alginic acid were higher and more reproducible than those obtained by accepted methods.

THE objective of the work reported here was to devise an effective procedure for determining the uronide content of pectic and other polyuronide substances in an appreciably shorter time than that required to carry out this type of analysis by currently recommended modifications (*1–7*, *9–15*) of the Lefevre-Tollens (*8*) method. Whereas a minimum time of 4 hours is considered necessary for complete decarboxylation of certain pectic materials with boiling 12.5% hydrochloric acid and even a longer treatment does not suffice for alginic acid, quantitative yields of carbon dioxide were obtained from a variety of pectic substances and alginic acid when they were heated in 19% hydrochloric acid, at a bath temperature of 145° C. for 1.5 and 2 hours, respectively.

APPARATUS AND METHOD

The apparatus used in the investigation is shown in Figure 1. Two assemblies of this type were used simultaneously in the same heating bath.

Air, the carrier gas, passes through an Ascarite or soda-lime column, *A*, which removes traces of carbon dioxide. A mercury valve, *B*, allows gas to pass in one direction through the apparatus. This valve is connected through a side tube, *C*, to a reaction flask, *D*, by means of a rubber connection. *D* is a 100-ml. round-bottomed, long-necked boiling flask, with a 24/40 ground joint attached. The oil bath, *E*, is maintained at 145° C. by means of a thermoregulator and immersion heater.

From the reaction flask the carrier gas passes upward through a 20-cm. reflux condenser, *F*, through a trap, *G*, containing 25 grams of 20-mesh granulated zinc or tin, and finally into the absorption flask, *H*. This 250-ml. Erlenmeyer flask is equipped with a 24/40 ground joint and a side tube attached a little below the ground joint, as shown.

The gas passes upward through an absorption tower, *I*. The lower part of the tower consists of an 18-mm. tube, fitted with a medium fritted Pyrex disk and sealed to the lower end of the inner part of a 24/40 ground joint. The bubbling disk should terminate 1 or 2 mm. above the bottom of the absorption flask when the joint is in place. A bulb of approximately 100-ml. capacity is blown above the ground portion of the joint to serve as a trap to prevent the possible loss of alkali by foaming. The outer portion of a 24/40 ground joint is sealed on above this bulb. The absorption tower, from the bottom of the disk to the top of the ground joint, is approximately 30 cm. in length. The top of the tower is fitted with a hollow, ground stopper, with a short side tube attached. The carrier gas passes from the tower assembly to a soda-lime tower, *J*. A water pump, attached to a capillary-tube regulator, *K*, serves to sweep 1700 to 2000 ml. of carbon dioxide–free air per hour through the apparatus during the heating period.

The sample to be analyzed is placed in the dry reaction flask. (The optimum size of sample used depends upon its uronic acid content—for example, about 250 mg. are sufficient for analyzing pectin.) Thirty milliliters of 19% hydrochloric acid and a small boiling tube are added. The ground joint is lubricated with sirupy phosphoric acid and attached to the reflux condenser and mercury valve. A stream of carbon dioxide–free air is drawn through the reaction flask and reflux condenser to remove traces of carbon dioxide before the absorption tower is attached.

The ground joints of the absorption tower are lubricated with stopcock grease and inserted into the absorption flask, *H*. The

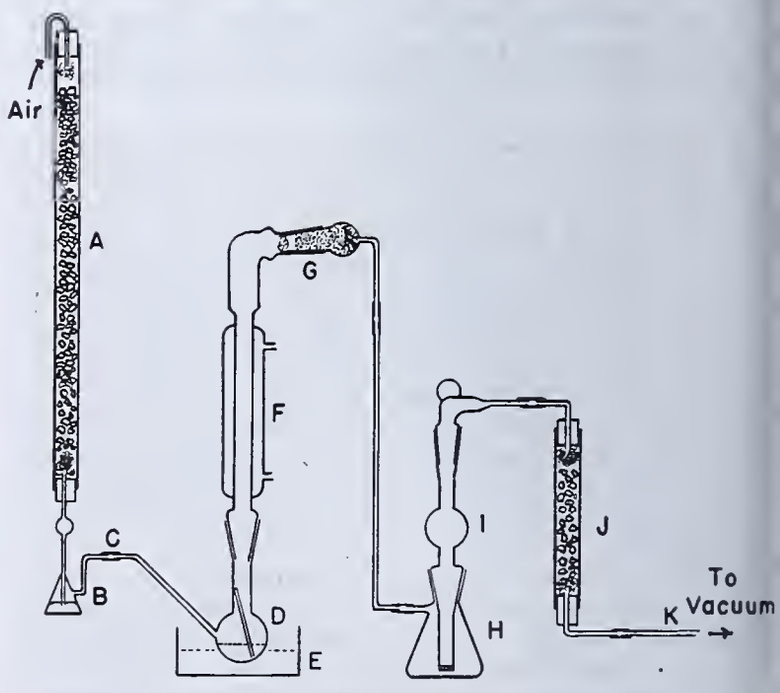

Figure 1. Apparatus for Determination of Carbon Dioxide Evolved from Uronic Acids by Heating with Hydrochloric Acid

flask and tower are swept free of carbon dioxide, and 25 ml. of 0.25 N sodium hydroxide and 5 drops of butanol-1 are added to the absorption tower. The side tube of the absorption flask is attached to the zinc trap and the top of the tower to the soda-lime tower, J, by means of rubber connections.

The oil bath, previously brought to 145° C., is placed in position, the level of the oil being 1 or 2 mm. below that of the liquid level within the reaction flask. After the initial rapid evolution of gas through the absorption tower has ceased, the capillary tube, K, is attached to J and to the source of vacuum.

Heating of the oil bath at 145° C. is continued while the apparatus is swept for 1.5 hours. The bath is then removed, the absorption flask and tower are disconnected from the apparatus, and the alkali is washed down from the tower into the absorption flask. Three or four washings are needed to remove the last traces of alkali from the tower. Gentle pressure with carbon dioxide–free air from the top of the tower can be used to hasten the washing.

Ten milliliters of 10% barium chloride dihydrate solution and two drops of phenolphthalein indicator are added to the absorption flask and the excess alkali present is titrated with 0.100 N hydrochloric acid. Normal precautions are used to exclude carbon dioxide during the storage, addition, and subsequent titration of the absorption alkali. A control standardization should be run without the sample and used in subsequent calculations of the carbon dioxide evolved from uronic acids.

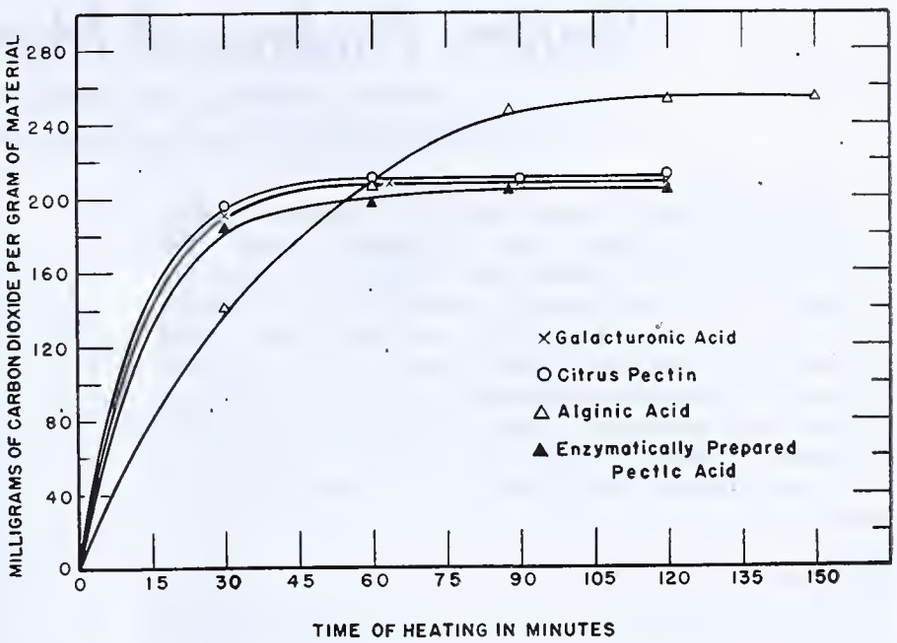

Figure 2. Rate of Evolution of Carbon Dioxide from Citrus Pectin, Galacturonic, Alginic, and Pectic Acids with 19% Hydrochloric Acid in an Oil Bath Maintained at 145° C.

EXPERIMENTAL RESULTS AND DISCUSSION

The results are presented on a moisture- and ash-free basis. Moistures were determined by drying in vacuo for 24 hours at 70° C., and dry samples were heated 3 hours at 600° C. to determine ash. The sample of galacturonic acid monohydrate was dried at 25° C. over phosphorus pentoxide and analyzed without further treatment.

Figure 2 shows the rates of evolution of carbon dioxide from galacturonic acid monohydrate, citrus pectin, alginic acid, and enzymatically prepared pectic acid with 19% hydrochloric acid in an oil bath maintained at 145° C. Pectin and galacturonic acid yielded the calculated quantity of carbon dioxide in one hour. Pectic acids required heating periods of 1.5 hours, while alginic acids required 2 hours for decarboxylation.

Table I shows results obtained with 12.5% hydrochloric acid at an oil bath temperature of 145° C. for 1.5 hours and 12.5% hydrochloric acid at an oil bath temperature of 130° C. for 5 hours, respectively, as compared with the proposed method with 19% hydrochloric acid and an oil bath temperature of 145° C. for 1.5 hours.

Table I. Yields of Carbon Dioxide from 0.250-Gram Samples of Uronic Acids and Their Derivatives

(Heating with 12.5% hydrochloric acid at 130° C. for 5 hours and at 145° C. for 1.5 hours, compared with 19% hydrochloric acid at 145° C. for 1.5 hours)

Sample	Yield of Carbon Dioxide, %: 12.5% HCl, 1.5 hours[a]	12.5% HCl, 5 hours	19% HCl	Calculated[b]
Galacturonic acid (monohydrate)	...	20.7 20.7	20.7 20.7	20.75
Citrus pectin (purified)	16.0 16.8	20.7 20.8 21.1	20.8 20.8 20.8	...
Citrus pectin (unpurified)	...	16.5 16.3	16.6 16.7	16.9
Apple pectin	...	21.4 21.2	20.7 20.8	21.2
Pectic acid (enzymatic)	14.5 14.4	20.0 20.0	19.8 19.8	20.0
Alginic acid (purified)	14.2[c] 14.6[c]	21.7 19.2 20.7	23.7[c] 23.8[c] 23.8[c]	24.3
Glucose	...	0.26 0.33	0.22 0.26	0

[a] Heated at bath temperatures of 145° C.
[b] Calculated from equivalent weight corrected for combined ash constituents and methoxyl groups.
[c] Samples heated for 2 hours.

Decarboxylation of citrus pectin (purified), pectic acid, and alginic acid with 12.5% hydrochloric acid at an oil bath temperature of 145° C. for 1.5 hours was approximately 70, 80, and 60%, respectively, complete. Equal quantities of carbon dioxide were obtained from the pectic substances with 12.5% acid for 5 hours and by the proposed method. However, the yields of carbon dioxide from alginic acid were low as compared with those obtained with the proposed method. The data clearly indicate the effectiveness of 19% hydrochloric acid over that of the lesser concentration and a very significant saving of time for uronic acid analyses.

The carbon dioxide evolved from various nonuronide substances such as inulin, gelatin, glucono-δ-lactone, starch, sucrose, and oxalic and mucic acids, when treated either under the reaction conditions herein recommended or those generally used, was similar to that obtained from glucose (Table I). A sample of arabinose yielded 0.7% carbon dioxide when analyzed by the proposed method. These results show that none of these substances would interfere in the analysis of pectic materials when present in moderate amounts as impurities. Quantitative recovery of carbon dioxide was obtained from citrus pectin when 0.020 gram of material was used instead of the recommended 0.250-gram sample.

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Routine Analysis of Manganese Bronze

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In a rapid method for the routine analysis of manganese bronze perchloric and nitric acids are used to dissolve the sample. The nitric acid is removed by fuming with perchloric acid, copper, tin, and lead then being precipitated as sulfides. Tin is determined gravimetrically, copper and lead electrolytically. Iron, having been reduced by hydrogen sulfide, is titrated with permanganate, after which the aluminum is determined by weighing the combined iron and aluminum oxides. Accepted methods are used in the determination of nickel and manganese. Results compare favorably with the certified values of two Bureau of Standards manganese bronze samples.

THE fact that relatively few methods for the complete analysis of manganese bronze have appeared in the literature is perhaps evidence that the inherent difficulties have not been satisfactorily overcome. It is known that if a sample of bronze containing considerable iron is decomposed with nitric acid the tin is incompletely precipitated, and contaminated with iron. Tin, if present in the electrolyte, will contaminate both copper and lead deposits if these elements are determined by electrolysis. Unless special precautions are taken the lead peroxide, deposited on the anode during electroplating, is contaminated with manganese dioxide. Also, the separation and determination of aluminum, at best being time-consuming, are complicated by the presence of any tin not previously separated. While accurate methods are available, all are objectionable for one reason or another. The principal objection is the time consumed in avoiding the interferences mentioned. Results of analysis are often needed in 5 or 6 hours, and most methods fail to satisfy this requirement. Some methods involve the use of fluorides to prevent interference from tin in the electroplating of copper and lead (*3*), but for large numbers of samples considerable platinum ware is required in subsequent evaporations. Procedures which require the plating of copper and lead without prior removal of manganese result in high lead values, although in some instances (*3*) the amount of contamination is not excessive for routine work. One of the most acceptable methods for determining aluminum necessitates the use of the mercury cathode (*2*), but is somewhat slow for rapid analysis.

The method described herein avoids these difficulties and is considered novel in some respects. The initial attack is made with perchloric and nitric acids, rendering soluble all constituents with the exception of a small amount of tin. Then, precipitation with hydrogen sulfide in the acid solution separates the copper, lead, and tin from the iron, aluminum, manganese, nickel, and zinc. By this means tin is removed from iron, and lead from manganese. The iron, having been reduced to the ferrous state by hydrogen sulfide, is titrated with permanganate. Later precipitation of mixed iron and aluminum hydroxides with ammonia makes the use of the mercury cathode unnecessary. The accuracy of the method is shown by values obtained with Bureau of Standards samples, and with a secondary standard which had been carefully analyzed by accepted methods (*1, 4*). Procedures for the determination of manganese and zinc are omitted because present methods (*5*) are satisfactory for the former, and the latter is most accurately obtained by difference.

PROCEDURE

All elements except zinc and manganese are determined from a single weighed sample. After the initial solution and sulfide separation, the precipitate and filtrate are treated simultaneously, in this way decreasing the total time of analysis. The complete procedure for determining tin, copper, lead, iron, aluminum, and nickel is as follows:

Transfer 1.0000 gram of sample to a 400-ml. beaker. Dissolve with 4 ml. of nitric acid (sp. gr. 1.42) and 6 ml. of perchloric acid (70%), cover, and heat until heavy fumes of perchloric acid have been evolved for several minutes. Cool somewhat, wash the cover glass, and dilute to 200 ml. with hot water. Pass hydrogen sulfide into the solution for 20 minutes. Coagulate the precipitated sulfides by adding paper pulp and bringing the contents of the beaker to boiling. Filter through a Whatman No. 30 paper into a 400-ml. beaker, and wash the precipitate thoroughly with hot water, combining the washings and the filtrate. Save both the precipitate and solution.

DETERMINATION OF TIN. Wrap the precipitate and filter in another piece of filter paper, and place in a clean No. 1 porcelain crucible. Ignite completely at 500° C., and, after allowing to cool carefully transfer the ignited material to a 150-ml. beaker. Pour 10 ml. of nitric acid (sp. gr. 1.42) into the crucible and heat on a hot plate for several minutes. Pour the acid into the beaker containing the major portion of the ignited oxides, and wash the crucible with 10 ml. of water, adding the washings to the beaker. Heat until the black copper oxide is dissolved. Dilute to 50 ml. with hot water, add a small amount of paper pulp, and allow to stand just below the boiling point for 30 minutes. Filter on a Whatman No. 42 paper into which has been poured a small amount of paper pulp suspension. Wash the precipitate three times alternately with hot water and dilute nitric acid (1 to 4). Ignite the precipitate in a tared crucible first at 500° C. until the paper is completely charred and finally at 900° C. until fully ignited. Weigh the ignited material as SnO_2, and calculate the percentage of tin.

$$\text{Per cent tin} = \frac{\text{weight of } SnO_2 \times 0.788}{\text{weight of sample}} \times 100$$

DETERMINATION OF COPPER AND LEAD. The filtrate from the tin determination contains the copper and lead. Dilute to 200 ml., and add 2 ml. of dilute sulfuric acid (1 to 1) and a small amount of urea or sulfamic acid. Place on an electroplater and deposit the copper and lead simultaneously. Using the customary gauze cathodes and rotating gauze anodes complete deposition will be obtained in about 60 minutes at 2.5 amperes. Weigh the previously tared electrodes and calculate the percentage of copper and lead. Discard the electrolyte.

$$\text{Per cent copper} = \frac{\text{weight of Cu deposited}}{\text{weight of sample}} \times 100$$

$$\text{Per cent lead} = \frac{\text{weight of } PbO_2 \text{ deposited} \times 0.864}{\text{weight of sample}} \times 100$$

DETERMINATION OF IRON. To the filtrate from the sulfide precipitation add a few glass beads, and boil the solution vigorously for 30 minutes to expel the hydrogen sulfide. Cool the solution in ice water, add 10 ml. of dilute sulfuric acid (1 to 1), and titrate with 0.05 *N* potassium permanganate to a faint pink color that should persist for 10 to 15 seconds. Reserve the solution for the determination of aluminum.

$$\text{Per cent iron} = \frac{\text{ml. of titration} \times 0.05584 \times \text{normality of } KMnO_4}{\text{weight of sample}} \times 100$$

DETERMINATION OF ALUMINUM. To the solution from the iron determination add 10 grams of ammonium chloride and make just alkaline to methyl red with ammonium hydroxide (sp. gr. 0.90). Add a small amount of paper pulp, bring to a boil, add 3 drops more ammonium hydroxide (sp. gr. 0.90), and allow to settle for 5 minutes. Filter the hydroxides on a Whatman No. 31 paper, washing three times with hot ammoniacal ammonium nitrate solution (10% ammonium nitrate made just ammoniacal to methyl red). Remove the beaker containing the filtrate and washings, reserving these for the nickel determination. Dissolve the hydroxides from the paper by pouring hot dilute hydrochloric acid (1 to 1) through the filter into the original beaker. Wash the filter well with cold water and discard. Add 2 grams of ammonium chloride to the solution in the beaker and reprecipitate the iron and aluminum hydroxides as described above. After

Table I. Results of Analysis of NBS Samples

	Sample 62a[a]			Sample 62b[b]		
	Found	Average	Certified	Found	Average	Certified
	%	%	%	%	%	%
Copper	61.47	61.47	61.51	57.36	57.35	57.40
	61.51			57.35		
	61.47			57.31		
	61.43			57.39		
	61.46			57.34		
Tin	0.84	0.84	0.84	1.02	1.01	0.97
	0.84			1.03		
	0.83			0.99		
	0.83			1.00		
	0.84			1.02		
Lead	0.51	0.52	0.50	0.28	0.28	0.27
	0.52			0.29		
	0.52			0.30		
	0.51			0.29		
	0.53			0.26		
Iron	1.02	1.03	1.04	0.80	0.81	0.81
	1.02			0.80		
	1.02			0.82		
	1.03			0.80		
	1.04			0.82		
Aluminum	0.94	0.93	0.92	0.98	1.00	0.97
	0.91			0.97		
	0.93			1.02		
	0.93			0.99		
	0.93			1.02		
Nickel	0.61	0.62	0.61	0.29	0.28	0.27
	0.62			0.27		
	0.61			0.29		
	0.62			0.29		
	0.62			0.28		

[a] Manganese 1.51%. [b] Manganese 1.28%.

filtering and washing the precipitate, place in a tared porcelain crucible, char the paper at 500° C., and ignite at 900° C. Cool the ignited oxides in a desiccator, and weigh the mixture of iron and aluminum oxide (R_2O_3) rapidly. From the weight of the R_2O_3 and the percentage of iron previously determined calculate the per cent aluminum.

$$\text{Per cent aluminum} = \frac{(\text{weight of } R_2O_3 - \text{weight of Fe} \times 1.429)\ 0.529}{\text{weight of sample}} \times 100$$

Determination of Nickel. To the filtrate from the first hydroxide precipitation add 15 ml. of dimethylglyoxime solution (1% alcoholic solution). Warm until the precipitate is well coagulated and filter through a Whatman No. 41 paper, washing eight to ten times with hot water. Wrap the filter in a moistened ashless filter paper and ignite in a tared, covered, porcelain crucible, first at 500° C., and finally at 800° C. Cool in a desiccator and weigh as NiO.

$$\text{Per cent nickel} = \frac{\text{weight of NiO} \times 0.786}{\text{weight of sample}} \times 100$$

EXPERIMENTAL RESULTS

National Bureau of Standards manganese bronze samples 62a and 62b were used to test the accuracy of the method. The results obtained are shown in Table I.

Most samples analyzed in this laboratory have less tin, manganese, lead, and nickel than do the Bureau of Standards samples. A sample of more common alloy, the composition of which had been carefully established by accurate methods (*1*, *4*), was analyzed by the above method. Results are shown in Table II.

DISCUSSION

The properties of perchloric acid make it particularly suitable for this method. Its oxidizing properties when hot and concentrated and the solubility of its salts provide practically complete solution of the sample. Further, the fact that dilute solutions of the acid have no oxidizing power makes it satisfactory for the hydrogen sulfide precipitation and iron titration. It was originally contemplated that the initial attack should be with perchloric acid alone. However, the tin values were found to be consistently low and somewhat erratic. It seemed possible that this might be due to volatilization of the tin during solution of the sample. By using both nitric and perchloric acids, and then evaporating to perchloric acid fumes, this difficulty was overcome. No error attributable to the presence of nitric acid was observed in the subsequent iron titrations.

Aluminum and tin values on sample 62b were found to be consistently high. Spectrographic analysis disclosed the presence of silicon in the ignited tin oxide and in the mixed oxides. This was due to the presence in the sample of 0.047% silicon which is not ordinarily found in manganese bronze analyzed in this laboratory. From a consideration of coprecipitation, hygroscopicity, and solubilities one might not expect to obtain the accuracy shown. However, it is possible that fortuitous compensating errors result in slightly better values than would normally be expected.

With this method one sample of manganese bronze can be analyzed in approximately 5 hours. Six samples require approximately the same time and 12 samples can be completed by one analyst in an 8-hour period. Experience with other methods had indicated that, in order to obtain the same degree of accuracy, about 8 hours are required for a single sample. The equipment required is found in all nonferrous laboratories, no special apparatus or reagents being necessary. All operations are of such simplicity that little is required in the way of special technique and training.

It has been demonstrated that a satisfactory analysis of manganese bronze can be accomplished if tin is separated from iron, if it is not present in the copper-lead electrolyte, and if manganese is separated from lead. The separations described in this method isolate the elements in such a manner that little interference is encountered in each determination. It therefore appears that the method at least partly satisfies the requirements for rapid analysis, and has sufficient accuracy to warrant its use in routine control and inspection work.

Table II. Results of Analysis of Laboratory Standard[a]

	Found	Average	Accepted Value
	%	%	%
Copper	56.03	56.01	56.01
	56.03		
	56.02		
	56.00		
	55.99		
Tin	0.36	0.37	0.37
	0.39		
	0.35		
	0.35		
	0.38		
Lead	0.26	0.24	0.21
	0.25		
	0.24		
	0.26		
	0.23		
Iron	0.82	0.83	0.82
	0.83		
	0.83		
	0.83		
	0.85		
Aluminum	0.64	0.63	0.61
	0.63		
	0.60		
	0.63		
	0.63		

[a] Manganese 0.42%.

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The views expressed in this paper are those of the authors and do not necessarily reflect the opinion of the Navy Department.

Determination of Small Amounts of Aromatic Hydrocarbons in Aqueous Solutions

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A method is described which employs formaldehyde–sulfuric acid reagent for the determination of small amounts (1 to 500 p.p.m.) of aromatic hydrocarbons in aqueous solutions with an accuracy of ±10%. The sensitivity of the test is 0.0001 gram of aromatic hydrocarbon.

THE determination of aromatic hydrocarbons in plant effluents or other aqueous solutions is a problem frequently encountered in laboratories. A reasonably accurate method for such analyses depends upon the development of a brown coloration when concentrated sulfuric acid containing a small amount of formaldehyde is brought in contact with an aromatic hydrocarbon under certain specific conditions.

Sulfuric acid containing formaldehyde has been used for detecting small amounts of benzene in air (*3*, *5*) a brown color being developed when the benzene-containing air is bubbled through the reagent. Toluene and coal-tar naphthas also give the test. Naphthalene is said to interfere by producing a black film. Thiophene and unsaturated hydrocarbons also interfere but can be removed by bubbling the air through sulfuric acid first.

An extension of this method has been reported (*4*, *5*), in which the degree of color development in the reagent is used in connection with a color standard to make a quantitative estimate of the amount of aromatic hydrocarbons present.

In the butanone method for benzene in air (*5*) the benzene is first nitrated to form *m*-dinitrobenzene, which is subsequently estimated colorimetrically with the aid of butanone. This method is both tedious and lengthy.

Another method (*5*) is also based on the nitration of benzene and subsequent separation of the *m*-dinitrobenzene from the nitrating acids by steam distillation. The amount of *m*-dinitrobenzene present is then determined by titration with standard titanous chloride solution. This method is also lengthy.

In the oxidation method for benzene in air (*1*, *2*) the sample is treated with hydrogen peroxide in the presence of ferrous sulfate, and the depth of the brown coloration which develops is taken as a measure of the amount of benzene present. Certain organic materials, particularly the more water-soluble solvents, interfere and must be removed before the test is carried out.

Of all these methods, the one employing the formaldehyde–sulfuric acid reagent seems to offer the best combination of speed, sensitivity, and ease of applicability to aqueous solutions containing aromatic hydrocarbons.

REAGENTS

Formaldehyde–sulfuric acid reagent, prepared by mixing 1.0 ml. of 37% U.S.P. formaldehyde with 100 ml. of C.P. concentrated sulfuric acid.

C.P. carbon tetrachloride (a technical grade may be used if it is washed with sulfuric acid until it remains colorless in contact with the reagent).

METHOD

The formaldehyde–sulfuric acid reagent is used as follows for determining the aromatic hydrocarbon content of aqueous samples: 100 ml. of the aqueous sample to be analyzed are shaken in a separatory funnel with 25 ml. of carbon tetrachloride. After separating, the carbon tetrachloride layer is carefully withdrawn and added to 5 ml. of the formaldehyde–sulfuric acid reagent in a 200-ml. flask. After vigorous shaking for 1 minute, the reactants are allowed to stand for 5 minutes, whereupon a brown discoloration of the acid layer develops.

The depth of this discoloration changes on standing; hence it is important that it be observed at some standard interval of time after shaking. The quantity of aromatic hydrocarbon present is estimated by comparing with a sample prepared at the same time and under exactly similar conditions from an appropriate quantity of an aqueous solution of known concentration of the aromatic hydrocarbon in question. For example, when styrene is being determined it is convenient to have at hand a standard solution of styrene in water. By trial and error the exact amount of the styrene solution of known concentration required to give a perfect color match with the unknown is determined. The styrene concentration in the unknown may then be calculated.

This procedure is actually applied to the determination of the styrene concentration in an aqueous unknown as follows: A standard styrene-in-water solution was made up containing 15 p.p.m. of styrene. Three trials were made in which (*a*) 5.0 ml. of the unknown were compared with 25 ml. of the standard, (*b*) 3.0 ml. of the unknown were compared with 50 ml. of the standard, and finally (*c*) 3.0 ml. of the unknown were compared with 60 ml. of the standard. The last mentioned gave a perfect color match between the unknown and the standard, so the concentration of styrene in the unknown was calculated to be $60/3 \times 15 = 300$ p.p.m.

SCOPE AND APPLICATION

Using the method outlined above, concentrations of aromatic hydrocarbons in water ranging from 1 to 10 p.p.m. may be determined with an accuracy of ±1 p.p.m. However, for higher concentrations it is necessary to dilute the sample with distilled water until it does fall within this range and then multiply the answer by a dilution factor. The inherent error of the method is multiplied by the dilution factor. Thus, for a sample containing 50 p.p.m. of an aromatic hydrocarbon, a fivefold dilution is recommended; and the final answer will be 50 ± 5 p.p.m.

Since the test is capable of detecting 1 p.p.m. of aromatic hydrocarbon in a 100-ml. aqueous sample, the sensitivity is actually 0.0001 gram of aromatic hydrocarbon.

The formaldehyde–sulfuric acid reagent has been applied to aqueous samples containing benzene, toluene, ethylbenzene, and styrene alone or in combination and in concentrations ranging from 1 to 500 p.p.m. It has been found that these aromatic hydrocarbons give different degrees of discoloration of the formaldehyde–sulfuric acid reagent; hence for the most accurate work it is necessary that the identity of the aromatic hydrocarbon be known. This is not necessary if only a rough estimate of concentration is desired.

For routine control work it was found desirable to have available a set of permanent color standards to eliminate the necessity of running a standard along with the unknown. Such a set of color standards was prepared by mixing appropriate quantities of colored inorganic salt solutions to obtain color matches with known samples containing 1, 2, 5, and 10 p.p.m. of aromatic hydrocarbon.

The partition coefficient of aromatic hydrocarbons between water and carbon tetrachloride is such that one extraction with carbon tetrachloride gives a complete removal from the water layer even in those cases where the aromatic content is very high.

Extraction reagents other than carbon tetrachloride may be used to remove the aromatic hydrocarbon from aqueous solution. (Dilution of the reagent prevents its direct application to aqueous solutions.) Diethyl ether results in a much lower sensitivity of the test; this very fact might be of value if the use requirements were such that high aromatic concentrations were frequently encountered.

EFFECT OF FORMALDEHYDE CONCENTRATION IN REAGENT

It has been recommended (*4*, *5*) that the formaldehyde concentration be 5 ml. of 37% formaldehyde per 100 ml. of concentrated sulfuric acid. In order to determine the effect of formaldehyde

ncentration, tests were made in which formaldehyde concenations of 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 ml. per 100 ml. of conntrated sulfuric acid were employed on solutions containing 2, and 6 p.p.m. of ethylbenzene. In all cases the low formaldeyde concentrations gave from two to three times the degree color development obtained with the strong concentrations. hus, for greatest sensitivity it is recommended that the formalehyde concentration be kept low. Obviously one formaldehyde ncentration must be accepted and maintained as standard to revent any possible variation from this source.

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Determination of the Solubility of Styrene in Water and of Water in Styrene

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e Karl Fischer reagent and cloud point observations have been pplied to the problem of determining the solubility of water in yrene between 6° and 51° C. The formaldehyde–sulfuric acid agent as well as cloud point observations were utilized for dermining the solubility of styrene in water between 7° and 65° C., d data are reported.

DATA on the solubility of styrene in water and of water in styrene and the effect of temperature on these mutual lubilities can be of considerable value when it is necessary to mpute organic losses by way of aqueous plant effluent streams. nce no such data are available in the literature, the present vestigation was undertaken.

SOLUBILITY OF WATER IN STYRENE

The Karl Fischer reagent (*2*) was applied successfully to the oblem of determining water in styrene. The data shown in able I and Figure 1 are erage values of two deterinations. The reproducility of the method is exllent, as the difference tween duplicate determiations never exceeded 3% the average value. Equirium conditions were obined by shaking a large mple of styrene containg a slight excess of water a given temperature and en allowing it to stand in water bath at this temrature for 24 hours to enre complete separation of e two phases before withawal of a sample of the yrene phase for titration of the water present with the Karl scher reagent. The styrene phase was always clear, and did not become cloudy even when cooled considerably from higher temperature at which saturation conditions had existed. loud points never occurred when an excess of both phases was esent.

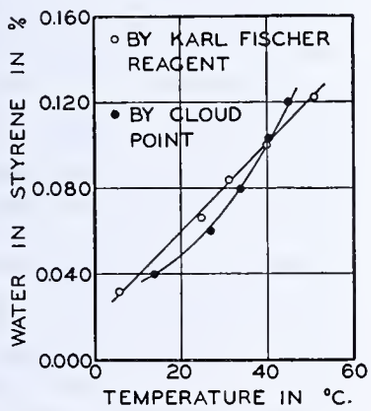

Figure 1. Solubility of Water in Styrene

Several precautions must be observed in applying the Karl ischer reagent as described above. Samples of styrene stored r several days over anhydrous calcium sulfate showed negliibly small blanks. Since styrene polymerizes readily, fresh mples were used for each determination. The usual polymeriation inhibitor, *p,tert*-butylcatechol, interferes even in the small amounts (10 p.p.m.) normally employed. The presence of this inhibitor in dry styrene caused rather high blanks, so styrene samples free of *p,tert*-butylcatechol were used in all the solubility determinations.

In spite of the fact that during the determination of the solubility of water in styrene by means of the Karl Fischer reagent no cloudiness or visual evidence of phase separation was ever observed (perhaps because of the presence of an excess of both phases), under slightly different conditions it was possible to observe a cloud point and to make use of this phenomenon for solubility determinations. The technique employed was the same as that described below. The data shown in Table I and Figure 1 are averages of two or more determinations. Duplicate determinations gave cloud points agreeing within 1° C., except in the case of 0.120% water in styrene, where the agreement was within about 3° C. Cloud points at 0.040% water were very faint.

Table I. Solubility of Water in Styrene

Temperature ° C.	Solubility of Water in Styrene %
	Karl Fischer Reagent
6	0.032
25	0.066
31	0.084
40	0.101
51	0.123
	Cloud Point
14	0.040
27	0.060
34	0.080
40	0.100
45	0.120

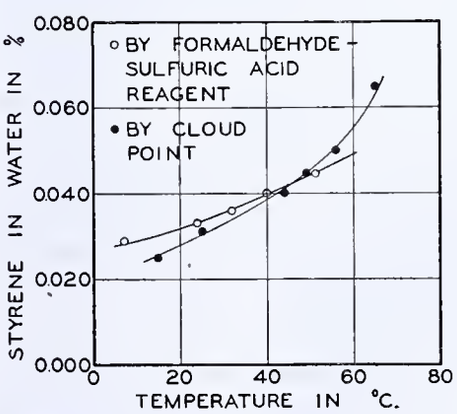

Figure 2. Solubility of Styrene in Water

SOLUBILITY OF STYRENE IN WATER

The solubility of styrene in water was determined by means of the formaldehyde–sulfuric acid reagent (*1*) and cloud point observations. (Table II and Figure 2). The value shown for 7° C. by formaldehyde–sulfuric acid reagent is the mean of three deter-

Table II. Solubility of Styrene in Water

Temperature ° C.	Solubility of Styrene in Water %
	Formaldehyde–Sulfuric Acid Reagent
7	0.029
24	0.033
32	0.036
40	0.040
51	0.045
	Cloud Point
15	0.025
25	0.031
44	0.040
49	0.045
56	0.050
65	0.058

minations. The one shown for 25° C. is the mean of two determinations; all other data are single determinations only.

During the course of the solubility determinations by means of the formaldehyde–sulfuric acid reagent it was observed that the system styrene-in-water exhibited a pronounced and seemingly sharp cloud point whenever the temperature was decreased from a higher value at which equilibrium conditions had existed. Use was made of this phenomenon to obtain further data on the solubility of styrene in water according to the following procedure:

A sample of styrene in water having a known styrene content was prepared and immersed in the water bath. The temperature of the bath was raised slightly above the temperature required for complete solubility of the styrene phase in the water phase and the sample shaken to ensure homogeneity. Then the bath temperature was allowed to decrease slowly and the temperature at which the first cloudiness of the sample occurred was recorded as the cloud point.

The cloud point exhibited by this system appeared to be very sharp, as the samples passed from a state in which they were clear to one in which they were cloudy within 1° C. The cloud point data shown in Table II and Figure 2 are averages of from two to eight separate determinations. Cloud points at 0.025% styrene in water were very faint.

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Excessively High Riboflavin Retention during Braising of Beef

A Comparison of Methods of Assay

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By comparison of values obtained with several variations in the fluorometric procedure for riboflavin as well as by comparison of fluorometric with microbiological values, no explanation could be found for the excessively high retentions encountered during braising of beef. Critical comparison of several current schemes for calculating cooking retentions in meats disclosed no fallacies in the method employed that could bring about falsely high results. It is suggested that there may be liberation of riboflavin during cooking from a precursor or complex of such a nature that neither the chemical nor the microbiological assays include it as "riboflavin" in the uncooked tissue.

THIS investigation grew out of the results obtained in a series of determinations of retention of riboflavin during the braising of beef roasts. In 19 such experiments on heel-of-round and chuck cuts, Tucker (*25*) had obtained an average total riboflavin retention of 110% with 15 of the retentions above 100%; the latter were in the range of 102 to 129%, with 12 above 105%. Such a trend was too pronounced to be dismissed as resulting from experimental errors in a fluorometric method which in 85 recovery determinations of crystalline riboflavin added to 31 different samples of raw beef, 31 samples of cooked beef, and 23 samples of beef drippings gave 76 recoveries between 90 and 105% with the remaining 9 between 83 and 111%.

It was possible, nevertheless, that some constant error in the method not exposed by recovery experiments, such as incomplete extraction, incomplete enzyme hydrolysis in the raw sample, or inadequate elimination of nonspecific fluorescent material in the cooked muscle or drippings, could account for the trend to excessively high cooking retentions. Furthermore, the method of calculating the cooking retention figures might be responsible for erroneously high results.

In the attempt to obtain evidence on these points two sets of experiments were carried out. In the first, two braising experiments compared results obtained by the fluorometric technique employed by Tucker (*25*) with those from four other fluorometric procedures, and with fluorometric and microbiological results obtained on the same samples by another laboratory. In the second, samples from a series of six braising experiments, including both chuck and heel-of-round cuts, were used to make more extensive comparisons between the fluorometric method originally employed and the microbiological method.

To consider the validity of the scheme of calculation used to figure cooking retentions, indirect evidence is here presented.

The fluorometric method which Tucker had used in her braising studies was a slightly modified procedure of that described for vegetables by Brush, Hinman, and Halliday (*3*), further details of which are reported under "Experimental Methods". It included a digestion of the samples with Clarase; adsorption on Florisil, originally suggested by Ferrebee (*7*) and Conner and Straub (*4*); oxidation of eluates with potassium permanganate as described by these authors; determination of individual eluate blanks made by exposure to light as suggested by Najjar (*15*) and applied to assays of cereals by Wright and Booth (*27*) and determination of "internal" standards as employed by a number of workers when determining fluorescence in digests directly (*8*, *13*, *16*, *19*) or in eluates (*1*, *2*, *12*).

Since the question of adequate elimination of interfering materials which might either enhance or depress fluorescence readings was to be studied, it was decided to select four methods which combined purification steps such as adsorption and/or pe

manganate oxidation with various procedures for preparing blanks. The methods chosen for comparison were two developed by Andrews *et al.* which, like the original method (Method 1), employed Florisil adsorption but differed either by omission of the oxidation step (Method 2, *2*) or by its application before adsorption (Method 3, *1*), as well as by use of sodium hydrosulfite to prepare blanks; and two which did not include adsorption but varied from each other in the steps for purification of digests and techniques for blanks. These were the procedures of Peterson, Brady, and Shaw (Method 4, *16*) and of Kahler and Davis (*9*) adapted to beef by McLaren, Cover, and Pearson (Method 5, *13*).

In making these comparisons in which the fluorometric techniques per se were under scrutiny, identical digests were used, proceeding separately after enzyme digestion and clearing by centrifugation. Preliminary comparisons had shown no increase in riboflavin values—that is, better extraction—on conducting the acid extraction at 100° instead of 70° C. (used in all earlier work as well as in these experiments), nor had they shown improved extraction or hydrolysis of bound riboflavin when papain and takadiastase instead of Clarase had been used for enzyme digestion. This is in agreement with the results of Peterson *et al.* *16*) on pork products.

On the other hand preliminary comparison of a 2-hour period 4) for Clarase incubation (0.4 gram per 100 ml., pH 4.5, and 8° C.) with "overnight" incubation had left no doubt as to the inadequacy of the 2-hour period, whether resulting from unadsorbability of "combined riboflavin" as convincingly suggested by Kosner, Lerner, and Cannon (*18*), from incomplete extraction, or from a less intensive fluorescence of some bound form. One of the latter two possibilities would seem necessary to explain the same type of result reported on pork by Peterson *et al.* when no adsorption was included. These authors adopted a 24-hour period for Clarase digestion, but in the present study a 20-hour period was chosen as the longest period which was compatible with continuing the assays on the day following the start of the analysis. However, in eight comparisons on older samples, either 20- nor 24-hour incubation yielded values consistently or significantly higher than the 16- or 18-hour values. In only one such comparison were definitely higher results obtained after the longer periods and they showed no advantage for the raw sample, since the results on the cooked one were increased even more than on the raw. It was tentatively concluded that in the earlier study the high cooking retentions could not have resulted from less complete hydrolysis of the raw than of the cooked muscle and that the 16- to 18-hour incubation periods then used were probably adequate for both.

In this study the case for complete extraction and hydrolysis rests on comparison with the microbiological results submitted by the Wisconsin group in the first experiment and more especially on the more numerous comparisons with microbiological values reported here as the second set of experiments.

EXPERIMENTAL METHODS

Cooking and Preparation of Samples. *First Experiment.* Two raw samples, the paired second rib chuck roasts, were taken from the same animal. The pieces taken for cooking were the contiguous first rib chuck roasts. One, No. 34, was braised without searing and the other, No. 35, was braised after searing. Each was cooked without seasoning until the internal temperature of the roast reached 93° C. By this arrangement it was hoped to determine whether there was any difference in uncooked chucks taken from the right and left side of the carcass as regards the riboflavin value per gram of fat-free dry weight—in earlier experiments on paired roasts one was analyzed raw and one cooked. In addition, evidence was sought as to whether searing might be a factor in bringing about high cooking retention results by fluorometric determinations.

All meat samples were ground twice and mixed with an electric mixer after each grinding to secure homogeneity. Samples of both the raw and cooked meat and of drippings were frozen and sent to the University of Wisconsin for microbiological determination.

Second Experiment. Four boneless chuck and three heel-of-round roasts were used. These had been braised and the raw and cooked parts prepared for sampling by Meyer for an investigation on pantothenic acid and niacin retentions in cooking of beef (*14*).

Calculation for Cooking Retention, Both Experiments. In order to calculate cooking retention it was necessary to determine the fat-free dry residues of all the samples. The fat-free dry weights were used to calculate the total original riboflavin content of the cooked roast in its raw state by the following formula:

$$\text{Total riboflavin before cooking} = \frac{\text{micrograms per gram (raw analysis)}}{\%\text{ fat-free dry weight raw}} \times (\text{cooked roast weight} \times \%\text{ fat-free dry weight cooked plus drippings weight} \times \%\text{ fat-free dry-weight drippings})$$

Cooking retentions were then based on the total riboflavin contents of each of the cooked parts in relation to the above calculated total before cooking.

Fat-Free Dry-Residue Determinations, Both Experiments. These were done in triplicate on raw and cooked meat and on drippings.

Meat. Approximately 10-gram samples weighed by difference from weighing bottles were spread over the walls of Alundum extraction shells and dried to constant weight (within 0.01 gram) in a vacuum oven at 95° C. and less than 100 mm. of mercury pressure for 6 to 12 hours. Then dry samples within the shells were extracted with ether for 40 to 48 hours in Soxhlet extractors.

Drippings. Approximately 10-gram samples of drippings were extracted three times with 10-ml. portions of ethyl ether by thorough shaking in weighed Maizel-Gerson (thiochrome) reaction vessels into which the samples had been weighed. After 5 minutes or more of centrifuging the ether-fat layer could be quantitatively removed with a capillary pipet each time. The water suspensions left were evaporated to dryness by immersion of the vessels in a boiling water bath and finally brought to constant weight in a vacuum oven. Thus the fat-free dry residues were weighed directly.

Preparation of Digests for Fluorometric Riboflavin Determination, Both Experiments. *Meat.* Digests for riboflavin determinations were made by blending 50-gram portions each of raw and of cooked ground meat with 300 ml. of 0.1 *N* sulfuric acid in a Waring Blendor for 3 minutes. Aliquots of these blends equivalent to about 13 grams of meat were weighed into digestion bottles (250-ml. centrifuge bottles) and the volume of acid was brought up to 200 ml. The digests were stirred with electric stirrers for one hour at 70° C., protected from light. They were then cooled and incubated with 0.8 gram of Clarase, making its concentration approximately 0.4% during incubation at pH 4.3 to 4.5 and 45° to 48° C. for at least 20 hours.

Drippings. Digests of the drippings were prepared by first quickly transferring with a wide-ended pipet about 10- or 20-gram aliquots from well-shaken suspensions kept at about 50° C. to weighed 50- or 100-ml. volumetric flasks, respectively, and weighing these after cooling. Thirty or 60 ml. of 0.1 *N* sulfuric acid and sufficient sodium acetate plus Clarase were then added to bring the pH and Clarase concentration to about the same level as used for muscle samples during incubation, with temperature and time the same for all.

Clarification of Digests of Meat and Drippings. Following incubation, 5 ml. of chloroform were added to each bottle or flask to dissolve the fat and take it to the bottom. Then each was brought up to volume (230 to 245 ml. for the meat digests and 50 or 100 ml. for the drippings), shaken thoroughly, and centrifuged to clear. The volume of digest was taken as the capacity of the bottle or flask minus 5 ml. for the chloroform, except that in samples of meat drippings in the second series of experiments, the complete flask contents were transferred to 50-ml. graduated centrifuge tubes and the volumes of the chloroform-fat layers thus determined were used as the corrections on total volumes.

The swelling effect of fat on the chloroform layer would be appreciable only in the case of drippings, and for the dripping samples used in the first series of experiments it was later estimated to be about 3 ml. Since these were made to 100-ml. volumes, riboflavin values of the drippings as determined in this series were probably about 3% too high, resulting in percentage figures for riboflavin retentions in the drippings too high by about 1% and therefore negligible so far as the total picture is concerned. The authors are grateful to E. E. Rice of Swift and Co. for bringing this error to their attention.

Up to this point all operations were carried out in a semidarkened room and the digests were protected from light as

Table I. Riboflavin in Clarase[a]

Sample No.	Method 1, Adsorption, $KMnO_4$ Oxidation, Light Blanks	Method 2, No Oxidation, Adsorption, $Na_2S_2O_4$ Blanks (10–20 Mg.)	Method 3, $KMnO_4$ Oxidation, Adsorption, $Na_2S_2O_4$ Blanks (10–20 Mg.)	Method 4, No Oxidation, No Adsorption, $Na_2S_2O_4$ Blanks (Solution 0.5 Ml.)	Method 5, Filtration at pH 6.8–7.0, No Adsorption, NaOH Blanks
	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.
A	2.2	8.5	..	..	..
B[b]	3.2	..	7.1	10.2	..
C	7.0	12.9	9.8	15.4	5.1

[a] Replicate determinations (2 or 3) on samples B and C were carried out in extracts of Clarase of concentrations near 0.013 gram per ml. Determinations on sample A and one additional series on sample C were done on extracts one fourth as concentrated. Average values of replicates are shown.

[b] Two microbiological determinations which were not in good agreement gave values of 3.6 and 4.4 γ per gram on this sample. No microbiological values were obtained on the other two samples.

much as possible. All subsequent operations were done in a dark room except when solutions were in brown bottles.

Fluorometric Methods. *First Experiment.* After centrifuging the digests, aliquots were taken for each method of analysis outlined below:

1. Adsorption, Potassium Permanganate Oxidation, Photolyzed Blanks. Aliquots of 25 ml. were adsorbed on 10- to 11-cm. columns of Florisil (2.0 to 2.3 grams). Elution with pyridine–acetic acid reagent (*4*) was carried to 45 ml. and duplicate 10-ml. aliquots plus 1 ml. of reagent were oxidized with 1 ml. of 4% potassium permanganate followed by 1 ml. of 3% hydrogen peroxide. The preparation of blanks and "internal" standards in separate qliquots as well as the photofluorometry were the same as those described by Brush *et al.* (*3*).

2. No Oxidation, Adsorption, Sodium Hydrosulfite Blanks. The adsorption and elution were the same as in Method 1 but Andrews' (*2*) method for treatment of eluates was followed with a modification of volumes. Three 10-ml. aliquots of the eluate were read, one with 1 ml. of added riboflavin solution for the internal standard, and the other two with 1 ml. each of added reagent. Blanks were obtained by reading each of these immediately after mixing with 10 to 20 mg. of crystalline sodium hydrosulfite.

3. Potassium Permanganate Oxidation, Adsorption, Sodium Hydrosulfite Blanks. The modified method of Andrews (*1*) was followed except that after the prescribed permanganate treatment of 25-ml. aliquots of digests, adsorption and elution were carried out as in Methods 1 and 2. Again 10-ml. instead of 14-ml. aliquots of the eluates were read. After addition of internal standards, volume corrections were adjusted to the volumes employed. Blanks were read without delay as in 2.

4. No Oxidation, No Adsorption, Sodium Hydrosulfite Blanks. The technique of Peterson, Brady, and Shaw (*16*) was followed exactly on 5-ml. aliquots of the beef digests.

5. Removal of Interfering Substances at pH 6.8 to 7.0, No Adsorption, Sodium Hydroxide Blanks. The technique described by McLaren, Cover, and Pearson (*13*) was applied to the Clarase digests of beef but otherwise followed exactly.

Preliminary to analyzing beef by the different methods, a digest of Clarase alone was made, using four times the concentration of Clarase usually employed in the meat digest, and replicate determinations of the Clarase blank values appropriate to each of the five fluorometric methods were made on this.

Second Experiment. The technique for fluorometric assays was the same as Method 1 in the first experiment.

Microbiological Assays. *Second Experiment Only.* The method of Snell and Strong as modified by Strong and Carpenter (*24*) was used, with one slight modification in preparation of neutralized filtrates of the digested samples. Although clear filtrates from the autoclaved digests were obtained at pH 4.5, the aliquots neutralized to pH 6.8 sometimes became slightly cloudy and, in one preliminary experiment, slight drifts were observed in microbiological values which were not eliminated by ether extraction. However, the solutions heated just to the boiling point at pH 6.8, quickly cooled, made to volume, and filtered, were clear again and gave microbiological values without drift. It was not necessary to treat drippings filtrates in this manner.

DISCUSSION OF RESULTS

First Set of Experiments. The results on Clarase alone are given in Table I. Sample C, used in this study, shows the same wide variation obtained by the several techniques in the riboflavin values per gram of Clarase as had been found in previous experiments with other samples of Clarase (A and B, older lots in use in this laboratory in 1942 and 1943, respectively) using several of these methods. Such results seem to indicate that some of these fluorometric methods are not measuring all the riboflavin or that others are measuring other fluorometric materials in the Clarase. Since such large differences are surprising, Table II is presented to show a typical series of fluorometric readings on sample C, from which the values per gram of Clarase have been calculated. When account is taken of appropriate blank corrections, internal standard readings, and dilution factors, the relation of these readings to final values is obvious.

To make direct comparison of readings in Methods 1, 2, and 3 possible, the readings of Method 1 are also shown calculated to the basis of 11 ml. for final volume. It can then be seen that two thirds of the difference between Methods 1 and 3 results from the different technique in preparing the blanks. Apparently some of the permanganate-stable and light-stable fluorescence of Clarase is not stable to sodium hydrosulfite and is therefore included as "riboflavin" in Method 3. Method 2 includes with the hydrosulfite-stable and with the hydrosulfite-unstable fluorescence some fluorescence which could be destroyed by permanganate, and Method 4 presumably further adds to these some which could be eliminated by adsorption. The readings in Method 4 obviously are not directly comparable to the other three, since not only are the volumes somewhat different but the riboflavin fluorescence is more intense in the buffered water extract than in the pyridine-acetic solution and the instrument was set at a lower sensitivity for these readings.

Further, comparison of "external" standard readings indicates that there is a marked "quenching" effect on the fluorescence of the "internal" (added) riboflavin in all the three methods in which the oxidation step is omitted: Methods 2, 4, and 5.

In the corrected readings for food eluates, however, variations due to Clarase are canceled out by the applica

Table II. Fluorophotometer Readings on Clarase Sample C[c] by Different Fluorometric Techniques

	Method 1		Method 2	Method 3	Method 4	Method 5
Digest represented in aliquot read, ml.	5.55[b]		5.55[b]	5.55[b]	5	5
Final volumes of aliquots, ml.						
a. Eluate or digest	13		11	11	10	15
b. Eluate or digest + 1 γ standard	13		11	11	10	16
c. Blank	13		11	11	10.5	16.05
Sensitivity setting of instrument, sodium fluorescein standard	70		70	70	55	70
Fluorophotometer readings[c]						
a. Eluate or digest	22.8	26.8[d]	39.1	29.5	66.1	67.0
b. Eluate or digest + 1 γ standard	52.5	61.9	70.0	65.5	98.2	89.4
c. Blank	7.9	9.1	10.5	4.4	34.1	54.2
Calculated						
Reading for riboflavin in eluate or digest ($a - c$)	14.9	17.7	28.6	25.1	31.3[e]	8.8[f]
Reading for 1 γ "internal" standard ($b - a$)	29.7	35.1	30.9	36.0	32.1	26.6[g]
Reading for 1 γ "external" standard under same conditions	30.0	35.4	36.3	36.3	43.9	34.0

[a] Concentration of Clarase in digest, 0.0128 gram per ml.

[b] 10/45 × 25.

[c] Not average but typical; all readings made with optical system suggested by Conner and Straub (*4*): Corning filter 511 only for incident light and No. 351 for fluorescent light.

[d] Calculated to 11-ml. volume for comparison with Methods 2 and 3.

[e] ($a - 1.05\ c$).

[f] ($0.937\ a - c$) for 5 ml. of digest in 16 ml.

[g] ($b - 0.937\ a$) for 1 γ in 16 ml.

Table III. Fluorophotometer Readings on Raw Beef Sample 35[a]

	Method 1	Method 2	Method 3	Method 4	Method 5
Fluorophotometer readings[b]					
a. Eluate or digest	28.7	40.4	37.3 (11 ml.)[b]	51.8	49.9
b. Eluate or digest + 1 γ standard	59.1	75.8	68.5 (12 ml.)	91.0	79.9
c. Blank	5.5	7.7	4.8 (12 ml.)	11.5	22.8
Calculated					
Reading for riboflavin in eluate or digest ($a - c$)	23.2	32.7	29.4[c]	39.7[c]	23.9[c]
Reading for 1 γ "internal" standard ($b - a$)	30.4	35.4	34.3[d]	39.2	33.2[d]
Reading for 1 γ "external" standard under same conditions	30.5	37.4	36.5 (12 ml.)	43.6	34.4
Correction for Clarase "riboflavin"					
On reading of aliquot of eluate	3.7	..	...	...	...
On calculated γ of "riboflavin" per aliquot of eluate or digest[e]	..	0.23 γ	0.17 γ	0.25 γ	0.083 γ

[a] Concentration of beef in digest, 0.0526 gram per ml.; of Clarase, 0.0032 gram per ml.
[b] Volume of digest represented in aliquot read, final volumes of aliquots, sensitivity setting of instrument, and optical system same as shown in Table II for readings on Clarase except for variation in final volumes in Method 3, indicated above.
[c] For Method 3 (0.917 $a - c$), for Method 5 (0.937 $a - c$), for Method 4 ($a - 1.05\ c$).
[d] For Method 3 ($b - 0.917\ a$), for Method 5 ($b - 0.937\ a$).
[e] Correction for Clarase not applied directly as reading correction in Methods 2, 4, and 5, because "quenching" effect of 4-fold concentration of Clarase was greater (Table II) than "quenching" in beef digests with 1/4 as much Clarase content. In Method 3 correction also made in γ because of inadvertent variation in final volume in this experiment.

tion of the proper "riboflavin" correction for Clarase in each type of assay. The nonriboflavin fluorescence from the beef itself, at least in eluates, is not so large or so variable by the various techniques. Table III shows a typical set of fluorometric readings on a raw beef sample, No. 35, with appropriate Clarase corrections.

The riboflavin values for beef and drippings did not vary greatly by the different techniques. Table IV includes the average values obtained in this laboratory from four determinations, two on the fresh samples and two after 3 weeks' frozen storage, by each fluorometric method, as well as single microbiological and fluorometric values obtained by the University of Wisconsin laboratory on the frozen samples. [McIntire *et al.* (*12*) have reported the methods of assay in use in that laboratory at about this time. Their fluorometric technique was essentially like Method 3 in these experiments.]

To check for the possible loss of riboflavin and for the adequacy of these methods as a whole, single recovery determinations were included for each sample by each method studied (Table V). Except for Method 1, there was a wider variation in the percentage recovery of riboflavin by these different methods than is desirable, but the lower recoveries were not limited to the methods including adsorption. Good recoveries in analyses of canned vegetables and fruits (*3*) as well as fresh vegetables have also been obtained by this method (*22*, *26*).

From Table IV, it is noted that for both raw and cooked muscle samples, the method including no oxidative step nor adsorption, but using a hydrosulfite blank, Method 4 (Peterson *et al.*), gave definitely higher results than all others. This might indicate that a small amount of nonspecific fluorescent material eliminated by hydrosulfite is included as riboflavin in the analysis of the muscle samples by Method 4, and is eliminated by adsorption in other methods. This seems to be a safe deduction because the standard recovery tests do not indicate an excessive loss of riboflavin itself during adsorption. In Method 5 the use of alkali for a blank determination would seem to account for the difference from Method 4, since no appreciable precipitation was obtained at pH 6.8 to 7.0, and this filtration step thus would hardly seem responsible for removing interfering materials. However, if extra fluorescent materials are so included in Method 4, they seem to be reasonably proportional in cooked and raw samples, since cooking retentions in the roast (Table VI) obtained by using Method 4 are in line with the others.

In this experiment, although there were consistently higher retentions of riboflavin found in both seared muscle and drippings as compared to unseared ones in all fluorometric methods (except in drippings by the Wisconsin fluorometric method), it would be unreasonable to ascribe this trend to extra nonspecific fluorescent substances in the seared, since the microbiological values show a comparable difference in the same direction. Although the two roasts were presumably almost alike in riboflavin content before cooking, judging from the results on the paired raw cuts (Table IV), and although application of the "*t*" test indicated that the difference between the riboflavin retentions of the seared and unseared roasts, though small, is significant, caution must be used in assigning the cause to the searing itself. This seems especially important because in 12 other experiments on quicker methods of cooking beef, such as frying and broiling of steaks, extensive searing was carried out and yet retention of riboflavin exceeded 100% in only 3 instances with only one of these as high as 106% (*25*).

Thus, the comparison of fluorometric techniques exposed no distorted relationships between the assay results on cooked samples, seared or unseared, as compared to raw, and pointed to no solution to the problem of excessively high retentions of riboflavin in cooking.

Second Set of Experiments. The values obtained by both the fluorometric and microbiological assays for the riboflavin contents of four chuck roasts and two heel-of-round roasts are listed in Table VII, including results on raw and cooked muscles and on drippings. Excellent agreement is shown between the values obtained by the two types of assays, although a tendency to slightly higher values by the microbiological method is noticeable, especially in the case of drippings for the chuck roasts. In these, the difference reaches a maximum of 12%, but averages about 9%. The variations between the two assays on all the

Table IV. Comparison of Riboflavin Content in Beef Chuck Analyzed by Different Methods

Sample No.		Fluorometric Methods, Chicago Laboratory					Wisconsin Laboratory	
		Method 1	Method 2	Method 3	Method 4	Method 5	Fluorometric method	Microbiological method
		γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.
34	Raw	2.19	2.28	2.27	2.84	2.19	2.15	2.40
	Cooked (unseared)	2.27	2.47	2.30	2.85	2.24	2.40	2.42
							γ/cc.	γ/cc.
	Drippings	2.33	2.65	2.47	2.64	2.40	2.92	2.64
							γ/g.	γ/g.
35	Raw	2.07	2.25	2.17	2.67	2.31	2.45	2.20
	Cooked (seared)	2.38	2.47	2.43	2.95	2.40	2.81	2.55
							γ/cc.	γ/cc.
	Drippings	2.48	3.05	2.57	3.10	2.92	3.08	2.64
	Comparison of values on paired raw samples based on fat-free dry weight							
							γ/g.	γ/g.
34	Raw	10.53	10.96	10.91	13.65	10.53	10.34	11.54
35	Raw	10.40	11.31	10.90	13.42	11.61	12.31	11.06

Table V. Percentage Recovery of Crystalline Riboflavin Added to Beef and Beef Drippings[a]

	Method 1	Method 2	Method 3	Method 4	Method 5
	%	%	%	%	%
Range	88–101	80–121	81–110	79–116	76–126
Average	97	99	94	103	90

[a] Ranges and averages of six determinations, one for each sample of raw and cooked meat and drippings.

Table VI. Retention of Riboflavin during Braising

Sample No.		Fluorometric Methods, Chicago Laboratory					Wisconsin Laboratory	
		Method 1	Method 2	Method 3	Method 4	Method 5	Fluorometric method	Microbiological method
		%	%	%	%	%	%	%
34	Roast (unseared)	71	74	70	69	71	77	69
	Drippings	25	28	26	22	26	33	26
	Total	96	102	96	91	97	109	95
35	Roast (seared)	79	76	77	76	72	79	80
	Drippings	28	31	27	27	29	29	28
	Total	107	107	104	103	101	108	108

chuck meat samples are from 3 to 6%, making an average of about 5% by which the microbiological values exceed the fluorometric, both for raw and cooked samples. In the two heel-of-round roasts all assays show even closer agreement.

As can be readily deduced from these results, the picture of cooking retentions would thus be comparable by the two methods of assay, with any difference being in the direction of higher retentions in the drippings and thus slightly higher total retentions by microbiological assays (Table VIII).

In summary of the total cooking retention picture it can be said that in only one roast out of seven was the retention under 100%, whereas among the other six, ranging from 101 to 122%, four showed total retentions above 105%. These results corroborate earlier findings, that riboflavin retentions above 100% by a significant amount are frequently obtained in beef braising experiments. And since the microbiological assays give similar results, it can be concluded that earlier excessively high cooking retentions obtained in such roasts, with fluorometric analyses, were not due to inadequate extraction or hydrolysis of the raw sample.

SCHEME OF CALCULATION OF COOKING RETENTION

The method of calculating cooking retentions on the basis of total fat-free dry weight of the cooked roast plus drippings assumed no significant change in the weight of the fat-free dry residue during the cooking process. Moreover, the B vitamins were assumed to be present in amounts proportional to the fat-free dry weights in the original raw cuts. These assumptions are probably not strictly valid, but are considered to be less in error for the type of samples used in this study than the supposition that their original total vitamin contents were proportional to their original moist raw weights, implying that the raw cuts of meat contained the same proportion of fat. Moreover, in order to calculate an original raw weight of meat which is cooked while attached to a bone, it must be assumed that the weight of the bone did not change significantly during cooking. The assumption about the homogeneity of the original raw samples obviously would be more valid for finely divided pieces such as boneless stew meat and especially for lean pieces such as liver slices. It is the basis for the method of calculation in which total moist weights before and after cooking are employed and has been used by McIntire *et al.* (*10, 11, 12*) and in most instances by Schweigert *et al.* (*20, 21*) and by Rice and Robinson (*17*). Even though this type of calculation seemed less valid for the beef cuts used in this investigation, it was decided to determine whether its use would greatly change the retention picture. The results so calculated are listed in the last column of Table VIII and although they are lower on the whole, the four highest are still above 105%.

The original method of calculation was also considered as preferable, especially for this type of cooking, to that employed by Cover *et al.* (*5*) in dry roasting of beef rib roasts or that by Rice and Robinson (*17*) in the vitamin retentions during drying of beef. The former used simple ratios of vitamin values per gram of fat-free dry residues after cooking to those before cooking and the latter the same sort of ratio for vitamin concentrations per gram of protein. Such calculations involve the same initial assumptions as those included in the method which was adopted in this laboratory. They differ only in that concentrations of vitamins are dealt with rather than totals. Such a calculation should be entirely valid in methods of processing, like the ones reported in those papers, where no significant loss of fat-free dry residues occurs in the drippings. However, in these braising experiments 7.5 to 10% of the original fat-free solids were found in the drippings and, therefore, the method of "totals" is a more accurate method of calculation.

If the high retentions, in this present study, as obtained by both assay techniques were due to a fallacy in the method of calculation on the total fat-free dry-weight basis, it would seem that other vitamin retentions determined on the same meat and calculated by the same method should tend to be parallel, even though, because of destruction, they run below 100%. On this presumption, pantothenic acid and niacin data by Meyer (*14*) on the same beef cuts were compared and no correlations in per cent retention of the different vitamins on the identical samples were found.

Table VII. Riboflavin Values in Beef Roasts

Sample No.	Fluorometric			Microbiological		
	Raw	Cooked	Drippings	Raw	Cooked	Drippings
	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.
Chuck						
10	1.33	1.65	1.69	1.38	1.73	1.82
11	1.52	1.90	2.23	1.60	2.00	2.50
12	1.51	1.52	2.27	1.58	1.59	2.48
13	1.29	1.44	1.88	1.35	1.53	2.06
Av.	1.41	1.63	2.02	1.48	1.71	2.21
Heel-of-round						
1	1.69	1.98	1.75	1.72	2.01	1.77
3	1.58	2.08	1.90	1.57	2.17	2.00
4	..	..	..	1.83	2.30	1.85
Av. of 1 and 3	1.63	2.03	1.82	1.65	2.09	1.89

Moreover, in the frying and broiling experiments by Tucker (*25*), in which excessive retentions were not obtained, the calculations were made by the same scheme and the drippings, though small in volume, were also included.

Therefore, it would seem safe to conclude that the scheme of calculation is not responsible for the excessively high cooking retentions obtained in braising.

POSSIBILITY OF INCREASE IN ASSAYABLE RIBOFLAVIN DURING COOKING

Further support for the idea that the high riboflavin results sometimes obtained in cooking are not due to errors in the analytical procedures can be drawn from a few moderately high results reported but not always commented upon in the literature, most of them by microbiological assays.

Table VIII. Riboflavin Retentions in Braised Beef

Sample No.	Based on Total Fat-Free Dry Weights: Roast, Fluorometric	Roast, Microbiological	Drippings, Fluorometric	Drippings, Microbiological	Total, Fluorometric	Total, Microbiological	Based on Total Moist Weight: Fluorometric	Based on Total Moist Weight: Microbiological
	%	%	%	%	%	%	%	%
Chuck								
10	76	77	34	35	110	112	111	113
11	79	79	35	37	114	116	112	114
12	63	63	33	34	96	97	98	99
13	72	73	29	30	101	103	92	95
Heel-of-round								
1	78	78	26	26	104	104	99	98
3	80	81	39	41	119	122	115	114
4	..	81	..	26	...	107	...	106

McIntire *et al.* (*10*) report retentions of 106 and 105% in one sample each of veal stew and lamb stew and also (*12*) 108 and 109% in one sample each of broiled and braised beef liver including drippings, and Rice and Robinson (*17*) 107% in one sample of pork precooked for dehydration. These retentions were all based on total moist weights. Since, however, those reported by the first group are the exceptions rather than the rule, they could be considered to result from a combination of assay errors. Basing retention calculations on riboflavin values per gram of protein, the latter authors also report 109% for beef precooked for dehydration and 105% in the dehydrated beef, whereas Schweigert *et al.* (*21*) obtain 106 and 108% retentions in 2 out of 4 hams carried through the curing process. Rice and Robinson attribute their high retentions to the formation of stimulating substances (to *L. casei*) during cooking, whereas Schweigert *et al.* conclude that the best method of calculating the per cent of vitamin retention is on the basis of original and final weights of the samples tested because it is the most direct method. One wonders how much this conclusion was influenced by the fact that the alternative method of calculation, based on protein, brought these two riboflavin retentions and one niacin retention above 105%.

In the experiments here reported there was no indication of the presence of stimulatory material in the cooked samples, such as drift in microbiological assays. Standard recovery tests were also run with each microbiological assay and gave uniformly good results—96 to 98%. Moreover, one preliminary comparison of assays done with and without ether extraction of the filtrates from all three types of samples showed no alteration of values for any of them after ether extraction.

A recent report by Feaster *et al.* (*6*) shows that the phenomenon of increased riboflavin as determined by microbiological assay also occurs during storage of processed pork.

High retentions similar to those determined on beef have also been obtained in this laboratory in some cooking of vegetables assayed by the same fluorometric method.

Weinberger (*26*) reported 7 out of 13 determinations of retentions with several household methods of cooking cauliflower which fell between 109 and 129%, and 4 out of 10 on broccoli between 107 and 121%. Smith (*22*) has reported a few such high retentions in institutional boiling of carrots and green beans as well as in small-scale boiling of the latter. Another laboratory has likewise obtained such results on carrots using the microbiological method. Streightoff *et al.* (*23*) report an average riboflavin retention totaling 122% for boiled carrots plus boiling water in ten batches of stored miscellaneous varieties and of 112% in ten batches of fresh Chantenay Coreless carrots, during large quantity preparation. The senior author stated (personal communication), moreover, that the "data on riboflavin were very consistent" which must mean that a large majority of individual cooking experiments gave retentions above 110%, and added that they obtained "much the same sort of result in the case of cabbage".

In the light of all the above investigations it would seem that the probability of the liberation of riboflavin from a precursor or complex in certain types of cooking procedures must be given consideration. Moreover, since chemical and microbiological assays yield similar results, it would be necessary to postulate that the complex from which riboflavin is formed or released during cooking is of such a nature that the vitamin cannot be liberated by digesting or autoclaving under acid conditions as during the preparation of extracts, nor by digesting with Clarase in preparation for chemical assay, and also of such a nature that without release it is unavailable to microorganisms.

Long cooking as compared to short cooking should be further investigated and perhaps the possibility of an enzymic action in the tissue itself during a slow rise in temperature should be considered.

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Correction

In the article entitled "Quantitative Determination of Ergosterol, Cholesterol, and 7-Dehydrocholesterol" by Lamb, Mueller, and Beach [Ind. Eng. Chem., Anal. Ed., 18, 190 (1946)] an error was made in Table IV, where the antimony trichloride value on yeast sample 3 should have been 2.41.

Conductometric Titrations with Organic Reagents

Determination of Copper with Cupferron and Iron with Sulfosalicylic Acid

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The use of certain organic analytical reagents in conductometric titrations has been studied and cupferron found suitable for titrating copper in a sulfuric acid solution. The method determines copper rapidly in brass without removing tin and lead. Sulfosalicylic acid and its ammonium salt were used in the volumetric determination of iron. These compounds illustrate the use of organic reagents in titrations in which the change in conductivity is due to the formation of a soluble metallo-organic complex.

MOST of the analytical methods involving organic reagents are either colorimetric or gravimetric. In this work a study has been made of the use of organic reagents in conductometric titrations. Van Suchtelen and Itano (*21*) in 1914 titrated nitrate with nitron, calcium with oxalic acid, and a number of organic acids with barium acetate. Kolthoff and others (*6–8, 10, 16*) have used the amperometric titration method as a means of utilizing organic reagents in volumetric analysis.

Cupferron (ammonium nitrosophenylhydroxylamine) has been found satisfactory for the conductometric titration of copper. When a standard solution of cupferron is added to an acid solution of copper the very insoluble precipitate of copper-cupferron is formed and ammonium ions replace the copper ions in the solution. The reaction is

$$Cu^{++} + 2C_6H_5N(NO)ONH_4 = Cu[C_6H_5N(NO)O]_2 + 2NH_4^+$$

The release of the fast-moving ammonium ions causes a rapid increase in conductivity before the equivalence point is reached. After the end point, the combination of hydrogen ions with the excess cupferron causes a sharp decrease in conductivity. Typical titration curves are shown in Figure 1. Copper in brass can be titrated with a fair degree of accuracy without the removal of tin, provided the percentage of tin does not exceed 5%. Lead, zinc, nickel, and cobalt do not interfere but iron is precipitated with copper and causes high results.

The solution of cupferron is stable for at least 3 days if kept in a dark bottle. If allowed to stand for longer periods an appreciable quantity of nitrobenzene is formed which can be removed by pouring the solution through a filter paper. Germuth (*4*) has pointed out that the addition of 0.3 to 0.4 gram of *p*-acetophenetide to each liter of cupferron solution aids in its preservation.

APPARATUS

The apparatus consisted of an alternating current Wheatstone bridge arrangement with an electrically driven tuning fork and a Kohlrausch slide-wire. A Knight 7-watt amplifier, modified so that crystal earphones could be used, gives sufficient sensitivity of sound balance. The bridge is connected to the primary of a T-58A37 Thordarson transformer and the secondary of the transformer is connected to the phonograph plug in the amplifier. Apparatus similar to this is used for student conductivity experiments.

A more precise Leeds & Northrup conductivity measuring apparatus built around the Campbell-Shackelton shielded ratio box was used in checking the precision of the method, and a Coleman pH meter was used in following the change in hydrogen-ion concentrations in certain reactions.

The conductivity cell which easily accommodated 200 ml. was large enough so that no correction for dilution was necessary unless more than 10 ml. of titrant was used. Where it was necessary to add more than 10 ml., the corrected conductivity was obtained by multiplying the slide-wire ratio, $\frac{1000-a}{a}$, by the ratio of the final volume of the solution over the original volume.

The calculation of the actual conductance was eliminated by using the slide-wire ratio, $\frac{1000-a}{a}$, as the relative conductivity. Six readings, 3 before and 3 after the end point, were plotted against the number of milliliters of titrant used, and straight lines were drawn through the points before and after the equivalence point. The intersection of these two lines represents the equivalence point on the volume coordinate.

Some increase in precision was attained by calculating the end point according to the method described below.

END POINT BY CALCULATION

The inconvenience of determining the end point graphically can be avoided by a calculation similar to that of Boulad (*3*).

The equation for a straight line, $y = ax + b$, is used with the number of milliliters of titrant represented by x and the corresponding slide-wire ratio, $\frac{1000-a}{a}$, represented by y.

The slope, a, is obtained by picking two sets of readings before the equivalence point, which are far enough from the region of curvature to assure a straight-line relationship. The value of a is then used to calculate the constant, b, for each reading. An average of the b values is used with the value of a to make an equation for the line before the equivalence point. Similar procedure with readings after the end point results in another equation. Simultaneous solution of these two equations for x gives the volume of titrant at the equivalence point.

TITRATION OF COPPER WITH CUPFERRON

SOLUTIONS. Cupferron solutions were prepared by dissolving 30 grams of reagent quality salt in 500 ml. of water. The solution was kept in a dark bottle and its strength was checked against a standard copper sulfate solution about every 3 days.

Standard copper solutions were made by dissolving accurately weighed samples (about 4 grams) of electrolytic copper foil in nitric acid. After the addition of 4 ml. of concentrated sulfuric

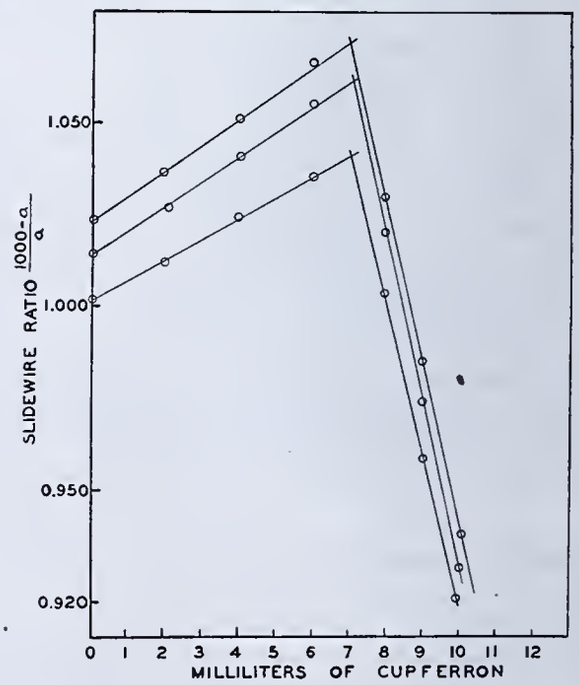

Figure 1. Cupferron *vs.* Bureau of Standards Bronze 52a

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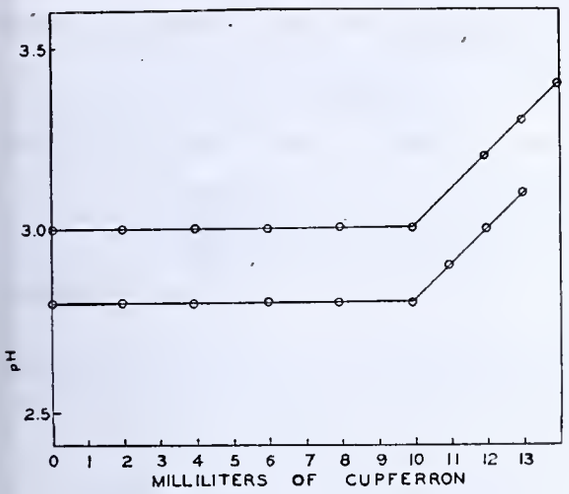

Figure 2. Cupferron vs. Bureau of Standards Bronze, Showing pH Change

acid the nitric acid was removed by evaporation to fumes, and the solution was diluted to 1000 ml.

PROCEDURE. Solutions of brass and bronze were prepared by dissolving weighed samples of William S. Murray brass samples and a bronze from the National Bureau of Standards, No. 52a, in nitric acid and proceeding in the same manner as with the standard copper solution. If the tin content of the brass or bronze was below 5%, no constituent or precipitate was removed. The bronze solution of Bureau of Standards 52a, which contains 14% tin, was filtered after the sample had dissolved in nitric acid. Other constituents of the brass or bronze were not removed.

A 50-ml. portion of the standard copper solution or of the brass or bronze was transferred by pipet to the titration cell and sufficient water was added to make approximately 200 ml. The cell was placed in a constant-temperature bath and the conductivity was measured. The standard cupferron solution was added from a microburet in about 2-ml. portions and the conductivity was measured after each addition. As the reaction between cupferron and copper is practically instantaneous, very little time is required for a titration.

Typical titration data, obtained by calculation, are plotted in Figure 1. The pH change at the end point is indicated in Figure 2. The appearance of this curve suggests that accurate pH measurements to three or four significant figures might be used to follow this reaction.

DISCUSSION

The time required for a titration was about 15 minutes and the calculation required about 5 minutes with a calculator, less time than the plotting of curves which would give the same accuracy.

The acid concentration is not a factor in the reaction; it was regulated to the extent that there was enough present to give a good curve after the end point and not so much that the conductivity became too high for measurement. The 4 ml. of concentrated sulfuric acid used in the preparation of the sample, with the subsequent dilutions, made a satisfactory acid concentration.

In accuracy the results show that the largest deviation is 0.8 mg. of copper per 100 mg. of copper taken, and that the average deviation is 0.3 mg. per 100 mg. of copper.

An attempt to analyze a bronze that contained iron, nickel, and antimony gave consistently high results.

The conditions of the analysis allow it to be fitted into the standard gravimetric procedure for brass.

Table I. William S. Murray Samples

No.	Samples Taken	Cu Taken	Cupferron Used	Cu Titer of Cupferron	Cu Found
		Gram	*Ml.*		*Gram*
33	2	0.1450	9.15	0.01583	0.1449
		0.1452	9.39	0.01551	0.1456
35	1	0.1245	8.56	0.01453	0.1243
36	2	0.1415	9.78	0.01453	0.1421
		0.1416	9.82	0.01453	0.1427
Bureau of Standards cast bronze 52a					
	4	0.0883	7.06	0.01258	0.0888
		0.0883	7.02	0.01258	0.0883
		0.0883	7.02	0.01258	0.0883
		0.0883	7.02	0.01258	0.0883
	2	0.0882	7.93	0.01115	0.0884
		0.0882	7.89	0.01115	0.0880

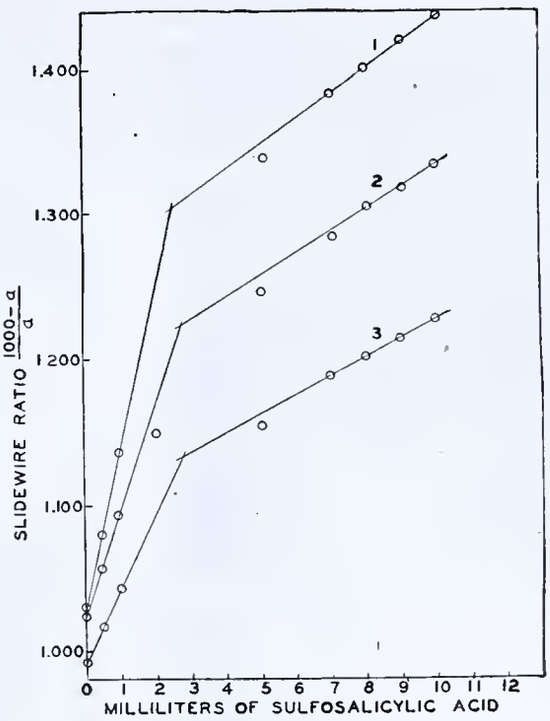

Figure 3. Sulfosalicylic Acid vs. Iron and Iron Ore

SULFOSALICYLIC ACID AND ITS AMONIUM SALT AS REAGENTS FOR IRON

Sulfosalicylic acid and its ammonium salt were found to produce usable curves in the titration of ferric iron (Figures 3 and 4). This acid has been used for separating iron from elements such as aluminum, manganese, magnesium, and others (*12–15, 17*) and in colorimetric determination of iron (*1, 2, 5, 9, 11, 18, 19, 20*).

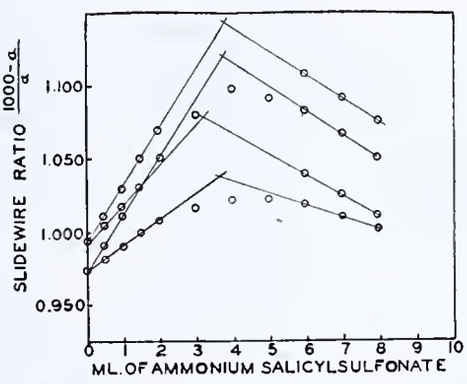

Figure 4. Iron vs. Ammonium Salicylsulfonate

Figure 3 shows the results when a 15% solution of sulfosalicylic acid, standardized conductometrically against iron wire, was used in determining iron in iron ore. Figure 4 shows the results when a standardized 10% solution of the ammonium salt of sulfosalicylic acid was used in place of the acid. The standard salt solution was prepared by dissolving the solid acid in ammonium hydroxide until acid to methyl red and titrating it conductometrically against standard iron wire. The calculated results in Table II show the accuracy of the methods. The iron solutions were prepared by the usual methods except that instead of reduction to the ferrous state, the iron was oxidized with either bromine water or Perhydrol to the ferric state.

SUMMARY

A method for the conductometric titration of copper in brass and bronze with cupferron is described, the accuracy of which

Table II. Sulfosalicylic Acid and Ammonium Salt of Sulfosalicylic Acid *vs.* Thorn Smith and Bureau of Standards Iron Ores

Sample	Iron Taken *Gram*	Titrant *Ml.*	Iron Found *Gram*	Difference *Gram*
15% Sulfosalicylic Acid, Iron Titer 0.0380 Gram per Ml.				
T.S. 72	0.0927	2.44	0.0927	0.0000
	0.1048	2.83	0.1075	+0.0027
T.S. 71	0.1072	2.85	0.1083	+0.0011
10% Ammonium Salt, Iron Titer 0.0247 Gram per Ml.				
B.S. 26	0.0813	3.36	0.0828	+0.0015
	0.1126	4.57	0.1128	+0.0002
	0.1126	4.56	0.1127	+0.0001
B.S. 27	0.1356	5.47	0.1351	−0.0005
	0.1356	5.46	0.1349	−0.0007
B.S. 29	0.0905	3.66	0.0902	−0.0003

varies from 0.2 to 0.8 mg. of copper per 100 mg. of copper taken. The method is fast and may be used without separation of certain constituents. The conditions of the titration allow it to be fitted into the usual gravimetric procedure for brass or bronze.

Methods for determining iron in iron ores are described which use both sulfosalicylic acid and its ammonium salt as titrating agents. The accuracy varies from 0.1 to 1.5 mg. per 100 mg. of iron taken.

The conductometric method may be used in applying the precipitation and colorimetric reaction of organic reagents to volumetric analysis.

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Determination of Tetraethyllead in Gasoline by X-Ray Absorption

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RIGID control of the antiknock rating of gasoline and other motor fuels for use by the armed forces is of utmost importance in maintaining high standards of performance. The use of tetraethyllead to bring various base stocks up to a set standard of quality requires the addition of varying amounts of lead to each lot of fuel. Continuous control of this process by the manufacturer and continuous checking by the user may involve the determination of the lead content of hundreds of samples per day in one laboratory. As a result, chemical methods of analysis for lead in gasoline have received a great deal of attention. No single chemical method has been proposed, however, which is generally applicable to the analysis of lead in chemically different base stocks, and which does not require an excessive amount of time for reasonably accurate results (*3*). Aborn and Brown (*1*) employed an x-ray absorption technique for the quantitative analysis of lead in gasoline, but their method was limited in sensitivity by the use of an ionization chamber to detect the radiation.

In the present work a Geiger counter was employed which had a quantum efficiency of roughly 80% for the radiation used. The result is a method for the quantitative determination of lead which is equivalent to the chemical analysis in accuracy, but may be completed by an unskilled worker in 5 minutes.

X-RAY ABSORPTION

Under the conditions necessary for the production of x-rays one observes a spectrum of continuous radiation upon which is superimposed a series of monochromatic characteristic radiations. The continuous spectrum (Figure 1) is composed of all wave lengths down to a certain minimum wave length given by $\lambda_0 = 12395/V$ Ångströms, where V is the peak voltage applied. The intensity of wave lengths longer than this minimum passes

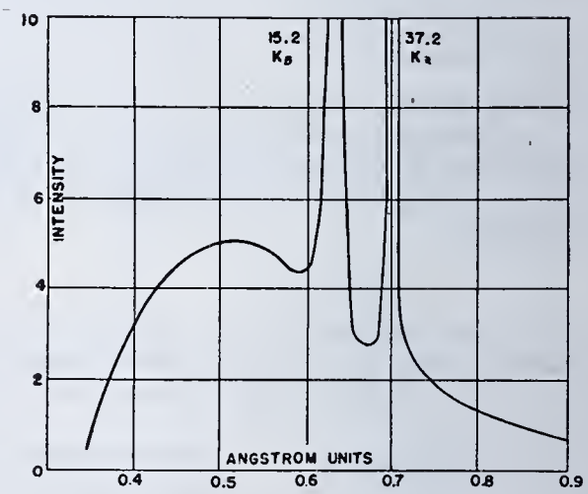

Figure 1. X-Ray Spectrum of Molybdenum Bombarded with 35-Kv. Electrons

through a maximum at about $\lambda = 1.3\ \lambda_0$ and then gradually approaches zero. The intensity of hard radiation in the continuous spectrum increases with atomic number of the target material. The characteristic K series radiations appear only when the peak voltage applied to the tube exceeds the K shell ionization potential of the target atoms. By the proper choice of target, voltage, filters, and possibly a crystal monochromator, almost any range of x-ray wave lengths or monochromatic radiation may be obtained.

When x-rays impinge on matter, the manner in which they are absorbed may be expressed by the relation:

$$I = Ie_0^{-\mu\rho x} \qquad (1)$$

where
I = intensity after passing through sample
I_0 = intensity of monochromatic x-rays incident on sample
μ = mass absorption coefficient
ρ = density of homogeneous sample
x = thickness of homogeneous sample

The mass absorption coefficient, μ, is a function of the wave length of x-rays and the atomic nature of the sample.

It is possible to express the mass absorption coefficient as a function of atomic number and wave length in the approximate relation:

$$\mu = (CZ^4\lambda^3)\ N/A \qquad (2)$$

where
C = a constant in the range between adjacent edges
Z = atomic number
λ = wave length of x-rays
N = Avogadro's number
A = atomic weight

At each critical absorption limit (corresponding to the ionization potential of the atom) the absorption coefficient reaches a maximum value on the short wave-length side of the limit. Where it is desired to identify one component of a mixture by absorption, the optimum contrast will be obtained by working with a wave length slightly shorter than the absorption limit for the particular component being measured. This is not always practical, since it is generally necessary to sacrifice intensity enormously to monochromatize the radiation. Because lead has a high absorption coefficient for practically all x-ray wave lengths compared to any organic material, no attempt was made to utilize an absorption edge in this particular analysis.

According to Equation 2 the mass absorption coefficient increases as the cube of the wave length. In a single-component system, therefore, one employs the longest wave length transmitted by the sample, thus obtaining a maximum change in intensity for a given change in sample thickness. However, leaded gasoline is a two-component system in which the mass absorption coefficients change at different rates as the wave length is varied. As a result an optimum wave length exists above which the advantage of using longer wave lengths disappears because the absorption coefficient for gasoline is increasing more rapidly than that of lead. With an absorption cell length of 15 to 25 cm. the optimum conditions were obtained with the x-ray tube operated at 17 kv.

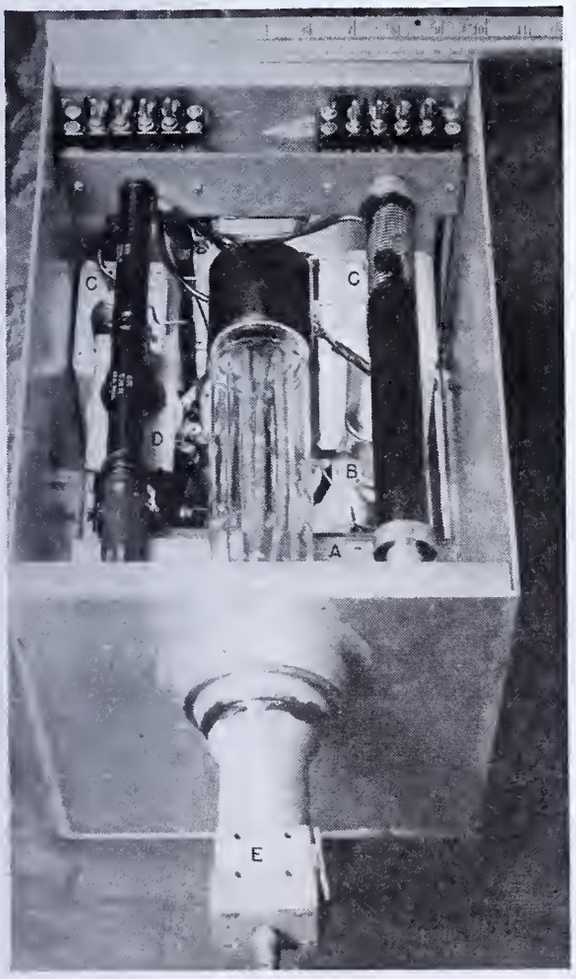

Figure 2. Internal View of X-Ray Power Supply

A. High-voltage transformer
B. 8013A rectifier
C. Capacitor
D. Filament transformer
E. X-ray tube

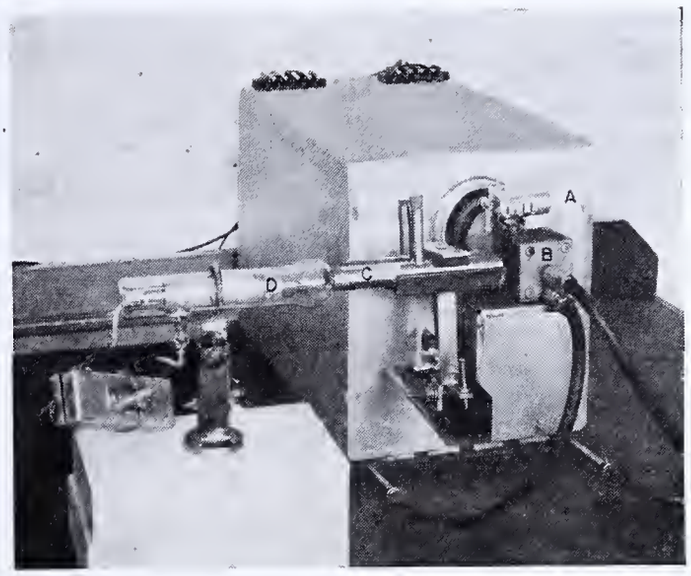

Figure 3. X-Ray Absorption Equipment

A. Power supply
B. X-ray tube
C. Absorption cells mounted on rocker arm for rapid comparison readings
D. Geiger counter

APPARATUS

The power supply (see Figures 2 and 3) consisted of a 25-kilovolt, 20-milliampere transformer, the output of which was half-wave rectified by an 8013 tube. A 0.05-microfarad condenser reduced the ripple below 10%. The x-ray and rectifier filaments were supplied by a single transformer with two secondaries. A molybdenum target Machlett x-ray tube of the line focus, water-cooled type was used. The entire supply including the x-ray tube was oil-immersed and occupied less than 28.32 liters (1 cubic foot). The power supply was stabilized by a line voltage regulator.

The x-ray supply and tube constitute a shockproof arrangement. The only safety hazard arises from exposure to the x-ray beam. The possibility of such exposure is minimized by ray-proof shielding, and the entire unit may be safely operated in an analytical laboratory.

The radiation detector was a Geiger counter tube developed by this laboratory. The success of the method depends largely on the fact that the Geiger tubes employed in this work were very efficient in the detection of soft x-rays. In its simplest form a Geiger counter consists of a cylindrical cathode and an axial wire anode, enclosed in a glass envelope containing a suitable gas mixture. A potential difference of the order of 1000 volts is applied across the electrodes in the manner illustrated in Figure 4. Under these conditions the passage of an ionizing radiation through the tube triggers a momentary discharge which may be detected as a voltage pulse at the capacitor, C. Following the discharge the counter quickly recovers to its initial condition and is ready to detect the next ionizing event. The high sensitivity of the counter is based upon the fact that a primary ionization of only a single ion pair is sufficient to trigger a momentary discharge of the order of 10,000,000,000 electrons.

In the range of wave lengths employed in these absorption measurements, x-ray quanta are absorbed almost entirely by photoelectric effect. The Geiger counters are constructed so as to permit passage of the radiation down the full 10-cm. length of the counter tube in the gas filling. Under these conditions, if a counter tube is filled with 40 cm. of krypton, 80% of the x-ray

beam at 17 kv. is absorbed photoelectrically in the active part of the counter. In other words, 80 out of every 100 quanta entering the counter produce counts.

The construction and operation of these tubes have been advanced to the point where their life is comparable to other tube elements in the equipment, and they are capable of from 10,000 to 100,000 counts per second, depending on the type of amplifier employed. A more detailed description of these tubes may be found in a previous publication (*2*).

The counting circuits used in the present equipment consisted of an amplifier capable of passing 25,000 random counts per

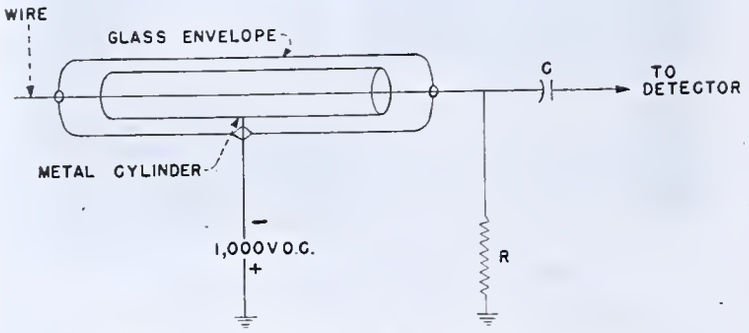

Figure 4. Fundamental Circuit of Geiger Counter

second coupled to a scale of 32, the output of which is registered by a mechanical impulse counter. In order to register 1000 random counts per second without missing more than 4%, the recording system must be able to count at least 25,000 uniform pulses per second. This is true because about 4% of the 1000 random counts arrive at an average rate greater than 25,000 per second. Since all measurements were made at a rate of well under 1000 counts per second, the recorded intensities were very nearly a linear function of the true intensity of radiation reaching the Geiger tube.

Recently the measurement of x-rays by means of a fluorescent screen and a multiplier phototube has been successfully applied in photofluorographic equipment by Morgan (*4*), and to the rapid examination of fuses by Smith (*5*). A comparison test performed at this laboratory showed that at x-ray intensities just sufficient to be observed above the background noise of the photomultiplier tube–fluorescent screen combination, the Geiger counter registered over 1000 counts per second.

EXPERIMENTAL

The unleaded gasoline used was standard aviation-base stock, 62-octane rating, and the fully leaded sample contained 4.6 ml. of tetraethyllead per gallon according to the accompanying chemical analysis. For the purpose of this work it was assumed to be correct and by careful dilution with the unleaded gasoline a series of samples was obtained ranging from 0 to 4.6 ml. per gallon in steps of 10%. The absorption cells were constructed of 15-cm. lengths of brass tubing (inside diameter, 0.5 inch, outside diameter, 0.75 inch) with 0.025-mm. (0.001-inch) aluminum windows.

In the optimum voltage range of 16 to 19 kv., and using a cell length of 15 to 25 cm., a ratio of intensities through leaded and unleaded gasoline of 140 to 1 was obtained. Counting rates of 75 to 1500 per second were registered when the tube current was about 5 milliamperes.

The ethylene bromide present in commercial ethyl fluid accounts for about one fourth of the absorption observed. Since a stoichiometric ratio of lead to bromide (or bromide-chloride mixture) is usually maintained in the ethyl fluid, this need not affect the accuracy of the measurements unless they are made on gasoline stocks whose history suggests that this ratio may have been altered. For special applications it may be desirable to work near an absorption edge of one of the components to minimize such interference. The present work covered only one base stock used with a particular ethyl fluid. Since the x-ray absorption coefficients for most base stocks are very nearly the same and much smaller than that of lead, a single calibration should be applicable to virtually all gasoline stocks using the same type of ethyl fluid.

In obtaining the calibration curve (a straight line), ten alternate 32-second counts were taken for each sample and averaged. Since the values plotted are ratios of counts obtained with clear gasoline to that of leaded gasoline, a total of 640 seconds or 10 minutes was required to locate each point. These results were obtained with an argon-filled counter. A counter filled with 40 cm. of mercury pressure of krypton was 6 times as efficient at 17 kv. and reduced the counting time correspondingly.

PERFORMANCE

By the method of least squares a calibration line was obtained which was more accurate than any one of the points, and by calculating the standard deviation of the points from the line (the square root of the mean of the squares of the delta y deviations from the line), an estimate was obtained of the precision of the determination. It was assumed in these calculations that all points have equal weight in the determination of the calibration line. This treatment is not rigorous, but a more exact treatment is not warranted. The standard deviation was 0.05 ml. of tetraethyllead per gallon of gasoline, or 1%. Similarly a precision of 1% was obtained with samples containing a maximum of 0.46 ml. of tetraethyllead per gallon. This corresponded to the detection of 0.005 ml. of tetraethyllead per gallon, or 0.0002% lead.

The precision attained here in the determination of lead in gasoline is comparable to that obtained by chemical methods. The x-ray absorption method is superior to the chemical method in most respects. It can be carried out in a routine manner by an unskilled operator in a small fraction of the time required for chemical methods. If conditions warrant it, the whole process can be made automatic for a number of simultaneous runs. The simultaneous exposure of the standard gasoline and the unknown will avoid the necessity for alternate exposures, eliminate errors due to fluctuations in x-ray intensity between successive exposures, and materially increase the precision.

OTHER APPLICATIONS

Although all measurements were made on leaded and unleaded gasoline, the method of analysis is applicable to lead in any other media of low atomic number. It will be possible to attain the same sensitivity for lead in any of the oils or fuels in common usage.

The method is not limited to heavy metals, however, because all elements have discontinuities in their absorption curves which may be utilized by careful control of the wave length employed to give maximum absorption for the particular metal desired. Further development of the technique may open up promising new fields for this method of analysis, many of which may be of great value in oil and fuel research. The testing of a good oil or fuel involves service runs in large mechanical units over extremely long periods of time. If a continuous method of analysis for metal particles could be employed, it is possible that "break in" and "break down" could be followed step by step. Wear rates of different metallic sections such as shafts and bearings could be selectively observed by the proper choice of radiation.

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Phenolic Resin Glue Line as Found in Yellow Birch Plywood

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A new technique for the analysis of phenolic resin glue lines is described. The method involves complete removal of the veneers by digestion with chromic acid, the glue being left in an unaltered state suitable for microscopic examination and subsequent stereophotomicrography. The relation of wood structure to the penetration of the adhesive is discussed with suitable illustrations.

PRODUCING stable physical and chemical properties in adhesive which bonds veneers is important at the present time, since it appears both desirable and possible to make strong wood structures possessing both variety in shape and diversity in size.

The fundamental theories of specific and mechanical adhesion were established to a considerable degree by the work of McBain and co-workers (*3*) in England and Browne and Truax (*1*) in the United States during and after the close of World War I. Although these and subsequent investigators have established the importance of specimen analysis strain test methods, and also the usefulness of cross-section analysis, they have left glue line thickness, the relative importance of specific and mechanical adhesion theories, and the variability of strength over limited areas, subjects of lively interest. These problems are discussed in the present paper in the light of work done with a new technique which permits examination of the surface features and structure of the phenolic glue line.

In order to carry out a comprehensive study of a plywood bond, factors such as glue line thickness, moisture content of veneers, gluing pressure, and temperature must be considered.

The glue line has been described as a continuous film of resin between two veneer surfaces and the thickness of this line has been correlated to the bonding pressure. McBain and Lee (*4*) and more recently Maxwell (*5*) claim that the strength increases with decrease of thickness. N. V. Poletika states that the strength varies inversely with the thickness (*6*). Browne and Truax (*1*) disagree with this view and say that the thickness of the film may vary within rather wide limits without affecting the strength of the joint.

The moisture contents of the veneers and of the resin before bonding are critical: when these are high, blistering results, and when low, the resin does not penetrate the superficial openings of the wood. In both cases the resulting adhesion is poor.

The bonding pressure employed depends on the flatness and density of the wood. The degree of polymerization depends on the temperature gradient and bonding time (*7*); hence the control of these conditions is an important factor in plywood manufacturing. The authors have been unable to find any reports relating all these factors with the nature of the resin bond under controlled conditions.

GLUE LINE EVALUATION BY SURFACE ANALYSIS

After careful examination of bleed-through resin removed from plywood by careful dissection it was shown that the resin was a true cast of the wood with which it was in contact. Chemical means were then employed for the removal of the wood. Hot chromic acid was selected as being most satisfactory for this purpose. This reagent has now been employed many times and has proved capable of digesting wood and not digesting phenol-formaldehyde resin within certain time and temperature limits.

The present standard practice is to suspend the test specimen in the hot chromic acid solution (100 grams of chromic acid in 100 cc. of water) with about 2.5 cm. (1 inch) immersed. The remainder of the specimen forms the support and carries the identifying code in notches on the upper edge. Digestion is carried on at 80° to 85° C. for about 20 minutes, which is sufficient to expose completely the glue lines in 3-ply $^1/_{16}$-inch birch veneers. After careful digestion, thorough washing with hot water is necessary to remove the chromic acid and the caramel-like products of oxidation, which, if present, obscure glue line detail. The specimen is then rinsed with acetone and allowed to dry. Though complete removal of this sludge is very difficult in the case of fiber casts, normal washing and drying are rapid and permit immediate examination of the resin.

The gross features of the glue line are examined with a Greenaugh twin objective microscope and areas of particular interest are stereophotomicrographed by a thoroughly tried method (*2*). During these observations and the subsequent photography particular attention is given both to those areas characteristic of a given kind of bonding and to those abnormal ones where failure is likely to occur first.

The real value of the technique depends upon the fact that high-power inspection with the microscope reveals the minute structure of the tracheae and tracheids. This inspection is carried out whenever evidence is required on the extent and thoroughness of the wetting by the unpolymerized resin of the wood.

For this examination the material is mounted in a medium of the same refractive index (normally about 1.66). Köhler illumination with narrow band filters complementary in color to the resin are employed.

MATERIAL EXAMINED

Many types of panels have been examined and their glue lines digested out, but a sufficient number of specimens from closely controlled experiments has not yet been examined to obtain a complete picture of the probable variability of the glue lines. Nevertheless, firm conclusions have been reached, illustrated by examples of glue lines from a set of panels made and tested by the Forest Products Laboratories at Ottawa, under controlled conditions.

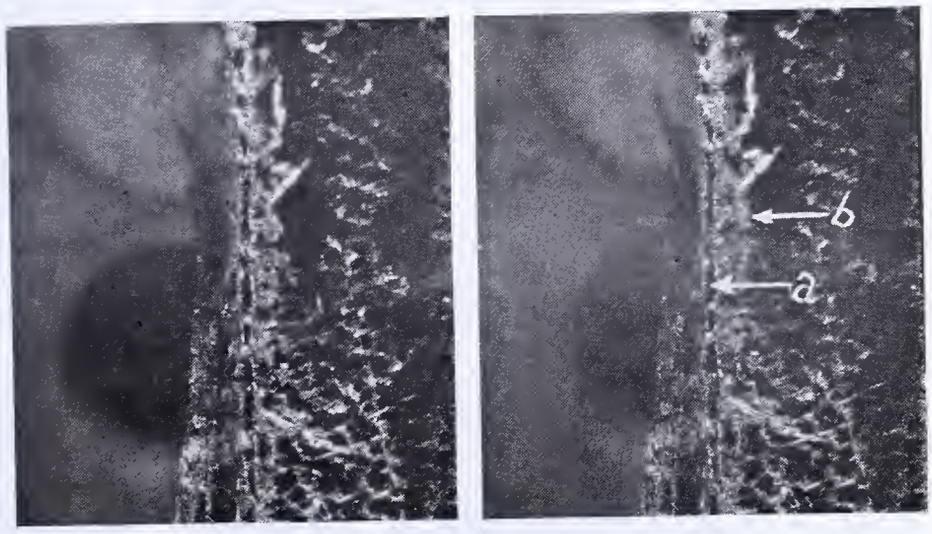

Figure 1. Low-Pressure Panel (× 7.5)

a. Large cavities are clearly visible in glue line. *b.* Very little penetration, as evidenced by hollow resin tendrils

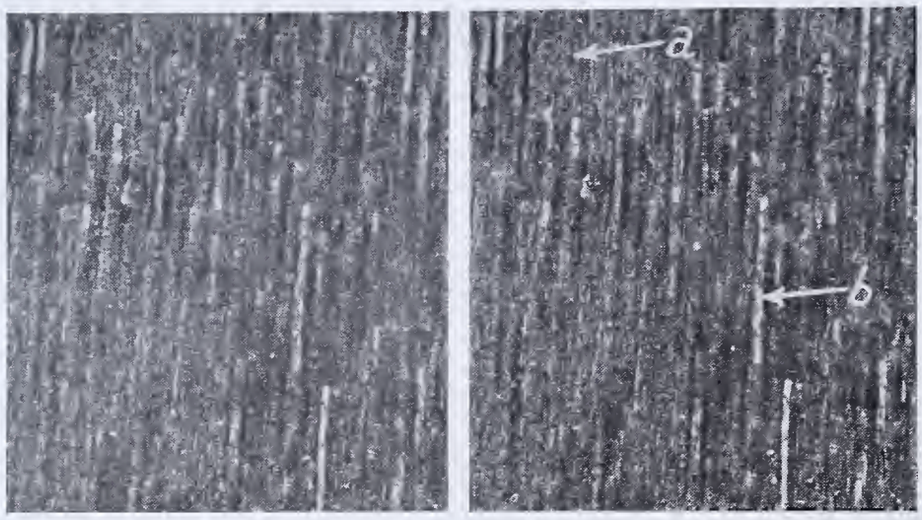

Figure 2 (*Left*). Normal Panel (× 7.5)

Stereophotomicrograph of resin glue line, showing how resin has filled all surface cavities, *a*, of veneer and penetrated into tracheae cells, *b*. Wood has been completely digested away.

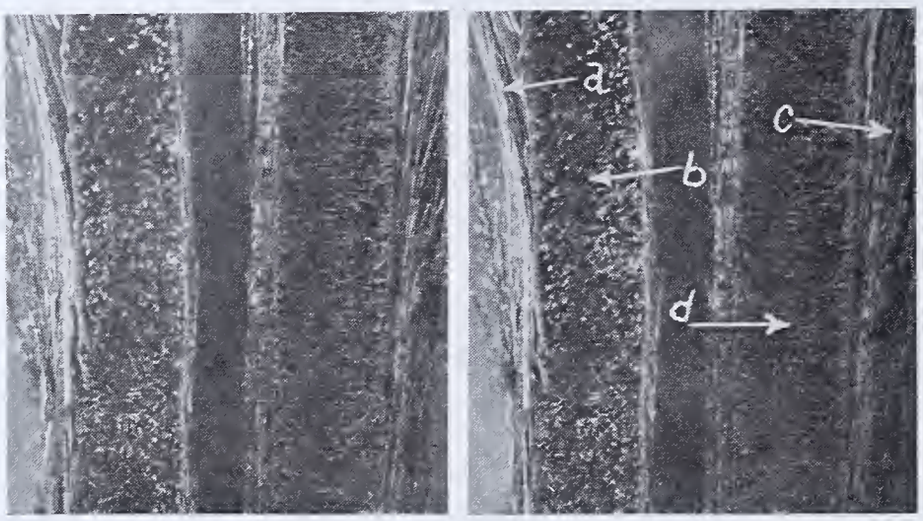

Figure 3. High Moisture Panel (× 7.5)

Stereophotograph showing end view of two specimens of plywood. Left specimen shows extreme penetration into one outside veneer, *a*, and center veneer, *b*. Right specimen shows extreme into one outside veneer, *c*, but almost no penetration into center veneer, *d*. This indicates extreme variation in penetration from point to point, as these two specimens were found within 1 inch of each other.

In all cases $^1/_{16}$-inch birch veneers and phenol-formaldehyde resin were used. The veneers were double-spread and the open assembly time was 1 hour. The veneers were bonded at 285° F. The bonding pressure, bonding time, and moisture content of the veneers were varied.

The panels were cut into 12 strips. Three tension normal to the glue line tests, and two shear tests were carried out on each of six alternate strips. The remaining six were subjected to the digestion treatment and examined microscopically.

LOW-PRESSURE BONDING

Figure 1 is a stereophotomicrograph of a typical edge, indicating the extreme thickness of the glue line and the noticeable lack of solid tracheal penetration present in a low-pressure bonded panel. This panel was made from veneers containing 9% moisture and was bonded under very low pressure for 8 minutes. The average value of the tension tests was 471 pounds per square inch. The shear test results were extremely low. Glue line failure occurred in almost every case.

Examination of the glue line from this panel showed that while wetting had occurred, there were open spaces in the glue line and that the casts formed within the tracheae were hollow tubes rather than solid rods.

OPTIMUM BONDING CONDITIONS

Figure 2 shows the solid character, the typically uneven thickness, and the intimate association of resin and wood which occurs in a good glue line. This illustration was made from a characteristic area of a 9% moisture panel bonded under 200 pounds per square inch for 6 minutes. The average value of the tension tests was 740 and the shear test was 195 pounds per square inch.

The peculiarities shown in Figure 2 were characteristic of not only the whole panel but also many other normal panels. The glue line was nonuniform in thickness, in most areas free from bubbles and composed of solid resin, in other areas discontinuous in nature. The resin had filled the broken surface cavities and passed into the open ends of the tracheae, tracheids, fibers, and medullary rays. The degree of penetration varied from place to place, depending on the orientation of the wood to the glue line and reached a maximum of about 5.0 cm. (Figure 4). Excessive penetration was ordinarily associated with starved areas. In these regions the glue line was very thin, broken, and frequently granular and the tracheae were usually just wetted, leaving thin-walled resin tubes.

HIGH-MOISTURE BONDING

Figure 3 shows a characteristic area in a panel made from veneers containing 14.9% moisture. They were bonded at 200 pounds per square inch for 6 minutes. The average value of the tension tests was 387 and of the shear tests, 132 pounds per square inch.

The very considerable penetration which occurred was characterized by complete wetting. The occurrence of large bubbles in the resin due to blistering resulted in the formation of tubes with thin broken walls. Because of the presence of steam or the ease of penetration under these conditions, the glue line was starved, thin, and discontinuous. When the orientation of the wood permitted, penetration to the surface occurred, and, as a result, produced bleed-through (Figure 4).

DISCUSSION

Many complete casts of the border pits present between tracheae in yellow birch have been found in spite of the fact that the pit diameter was of the order of $^1/_3$ micron (Figure 5). Though botanists believe that pits exist in the fiber wall, these either do not exist or else are too small for the flow of unpolymerized resin. However, many fiber casts have been obtained when the fibers were at the surface of the veneer and cut open during the cutting of the veneer. Wetting of the inner surface of the fiber is not often seen. It would seem that the presence of debris and air within the fiber produces an emulsionlike state which leaves the cast with a bubbly appearance and a rough surface (Figure 6). The surface characteristics of the resin trachea casts were identical with those of sectioned birch. The authors believe this evidence of the intimate contact of the resin and wood firmly sup-

Figure 4 (*Right*). Normal Panel (× 7.5)

ereophotograph showing extreme penetration into one itside veneer, *a*, and center veneer, *b*. Very little resin s penetrated into outside veneer, *c*. Photograph indicates variation in penetration due to wood structure.

orts the theories of both specific and me-hanical adhesion.

On the other hand, failure to confirm the iews of McBain and Lee, Maxwell and oletika, that there is a good negative corre-ation between the thickness of the glue line nd the strength of the bond, appears to be elated to the structure of the veneers sed in making plywood. The veneer surface, normally variable n character from point to point, is more or less upcrficially fractured, and possesses a chemical character differ-nt from metals, rubber, and plasties. These materials are omogeneous in a way in which wood is not, and it is reasonable hat the thinness and the strength of the bond should show a good egative correlation.

This technique permits the critieal examination of many kinds f phenol-formaldehyde glue lines. Striking variations in the lue line due to wood structure occur within areas smaller than 1 quare inch (Figure 3). The stereophotographs reveal that ellow birch is normally wetted and freely penetrated by the un-olymerized resin. Since general wetting is usually observed n both good and bad panels, it is eoncluded that certain factors ather than the variation in specific adhesion of resin to wood re involved in the variable quality of plywood panels. The elation of these factors to observed types of failure is discussed elow, although evidence is limited.

OBSERVED TYPES OF FAILURE

Normal Panel Failure. In panels where the glue line is elatively continuous and moderate penetration of resin into the adjoining vessels has oecurred, failure ordinarily takes place within the wood near the glue line. The failure at this point is believed to be related to the fact that the surface structure of the wood in all seetioned material is neeessarily damaged during cutting and that in badly cut veneers, the undamaged eore may not be very thick. It would appear that a panel bonded under normal eonditions cannot attain its maximum strength unless resin penetration has at least reached the undamaged core.

Low Pressure Failure. Examination of panels bonded under low pressure appears to indieate that failure is related to the decrease in contact area between resin and wood and especially to the fact that the wetted wood has probably been weakened during cutting. There is no indication that moderately thick glue lines are not strong. Cracked and brittle resin has not been indicated as a principal eause of failure.

High Moisture Content. Examination of high moisture bonded panels indicates that starvation is the essential cause of failure. The presenee of excess moisture results in distribution so extensive that only small amounts of resin are present in the critieal zone.

CONCLUSIONS

A new teehnique for phenolic resin glue line analysis permits the rapid analysis of glue line strueture without resorting to the laborious and time-consuming methods of section analysis. It avoids damage to brittle glue line structures which eannot be sectioned even when the best methods of softening are employed. To obtain a good bond the resin must penetrate below the surface, which is usually damaged by the cutting tool.

Observations indicate that adhesion cannot be attributed to

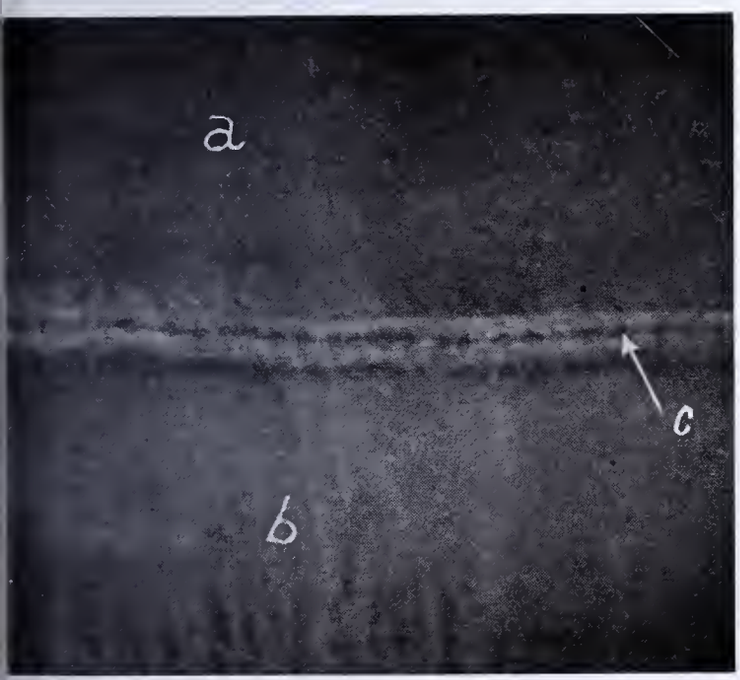

Figure 5. Resin Casts of Pits (× 1060)

Photomicrograph showing resin mold of two tracheae, *a* and *b*, and connecting pits, *c*

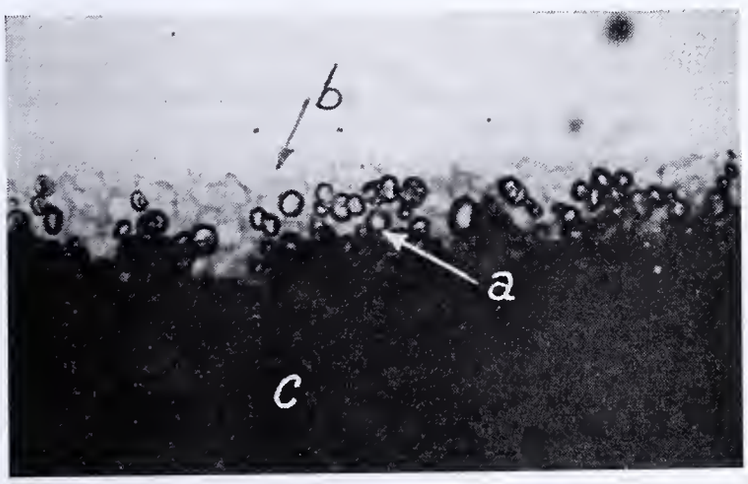

Figure 6. Fiber Casts (× 1240)

Photomicrograph showing granular structure, *a*, and rough surface, *b*, of fiber cast, *c*

either the specific or the mechanical theory but is dependent on the combination of the two.

ACKNOWLEDGMENT

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X-Ray Identification and Crystallography of Aldehydes and Ketones as the 2,4-Dinitrophenylhydrazones

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IN THE past few years a considerable amount of research has been undertaken to work out systematic and practical methods of organic analysis for carbonyl compounds using a solution of 2,4-dinitrophenylhydrazine (*2, 8, 13*).

The reagent is usually prepared by dissolving 2,4-dinitrophenylhydrazine in ethyl alcohol acidified with either hydrochloric or sulfuric acid. The addition of an aldehyde or ketone to such a solution usually results in the rapid formation of crystals of the corresponding 2,4-dinitrophenylhydrazone. If the aldehyde or ketone initially does not contain any great amount of impurities and if the derivative is carefully purified by recrystallization, the aldehyde or ketone undergoing analysis is identified from the color, optical properties, and melting point of the derivative.

When the unknown substance contains a mixture of aldehydes or ketones, the problem of identification becomes difficult since the melting point, which is the most widely used test, is so easily lowered by the presence of impurities. Furthermore, Brandstätter (*4*) has shown recently by thermal analysis the marked tendency of these hydrazones to form solid solutions with one another. Recourse is then usually taken to separation of the aldehyde or ketone by fractional distillation or crystallization before identification with the 2,4-dinitrophenylhydrazine reagent. Such a procedure is time-consuming and any improvement may be considered valuable.

Two instrumental methods have been suggested for identifying the derivatives of aldehydes and ketones which are affected to a lesser extent by the presence of impurities than is the usual method of melting point determinations. These are dependent upon optical crystallographic properties (refractive indices) of the derivative (*6*) and the x-ray powder patterns (*3*).

The major field of contention in the use of 2,4-dinitrophenylhydrazine as a reagent for the identification of aldehydes and ketones lies in the rather large number of modifications which have been reported from time to time. The exact nature of these modifications has caused much speculation. Two modifications of acetaldehyde–2,4-dinitrophenylhydrazone have been clearly shown to exist (*6*) as well as two modifications of derivatives of the furan series (*5*). Still other modifications of these and other compounds seem likely in view of the varied reports of crystalline properties and melting points (*1, 7, 12*).

[1] Obtained results on aldehydes. Present address, Tennessee Eastman Corp., Kingsport, Tenn.

[2] Obtained results on ketones. Present address, Shell Development Co., Emeryville, Calif.

The present x-ray diffraction investigation was undertaken, therefore, with the intention of clarifying uncertainties concerning these derivatives which are involved in this important method of identifying aldehydes and ketones, and of demonstrating the value of x-ray methods in many fields of organic analysis, both qualitative and quantitative, especially in series of related compounds of some complexity. In this paper are presented data on the unit cell dimensions and space groups of a number of aldehyde and ketone 2,4-dinitrophenylhydrazones, several of which are polymorphic; powder pattern data for rapid and certain identification by the organic analyst of aldehydes and ketones, together with techniques of handling micro or semimicro quantities of specimens; and a logical extension to quantitative analysis of pure and mixed aldehydes.

SINGLE CRYSTAL ANALYSES OF UNIT CELLS AND SPACE GROUPS

With single crystals which are ordinarily easily obtained, rotation, oscillation, and Weissenberg patterns have been made, the last type being especially valuable in this study; powder patterns were made in cameras specially designed and constructed for penicillin studies, in which the samples in cellulose acetate capillary tubes are rotated during exposure. Reciprocal lattice projections were made in all cases, but indexing of spots on the equiinclination Weissenberg patterns was easily possible by inspection of zones. All pertinent data including probable space groups on the derivatives of formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde, crotonaldehyde, furfuraldehyde, and dimethyl ketone are assembled in Table I. Typical procedures are illustrated with the case of the formaldehyde compound.

FORMALDEHYDE–2,4-DINITROPHENYLHYDRAZONE PREPARATION. The formaldehyde–2,4-dinitrophenylhydrazone was prepared by adding 40% formaldehyde solution to a hot concentrated solution of 2,4-dinitrophenylhydrazine dissolved in methyl alcohol approximately 2 *N* in hydrogen chloride. When cooled the crystals were filtered and air-dried, then recrystallized once from hot 90% ethyl alcohol and three times from hot isobutanol. The last recrystallization was slow and resulted in the growth of yellow needles and platelets about 2 to 5 mm. in length.

PROPERTIES OF THE CRYSTALS. The formaldehyde–2,4-dinitrophenylhydrazone crystals melted at 166° C. (uncorrected) agreeing with the literature (*6*). The density of the crystals as measured by floating in a mixture of methyl iodide and ethyl bromide was 1.592 ± 0.010 grams per ml., the density of the liquid being determined with a 25-ml. boot specific gravity bottle at 25° C. The needle-shaped crystals are similar to the platelets in being elongated along the *c* axis, the difference being in the

X-ray diffraction data are used to characterize 12 different crystalline aldehyde–2,4-dinitrophenylhydrazones from 6 different aldehydes and 16 ketone derivatives from 15 ketones using Weissenberg, rotation, oscillation, and powder patterns. This series was selected because of its importance in organic analysis of aldehydes and ketones; because there is no previous record of investigation outside of fragmentary optical data; and because it afforded excellent experience in extending the most modern techniques, particularly in distinction between polymorphic forms and in use of micro quantities of specimens. This study has involved the practical reciprocal lattice interpretation of the powder pattern of a triclinic crystal. Polymorphism is very generally observed in the series of hydrazones. Each is uniquely distinguished by its x-ray powder diffraction pattern far better than by color, habit, or melting point. A micromethod of qualitative analysis for pure and mixed carbonyl compounds is described. Aside from the necessary x-ray equipment the apparatus required is readily available. Ordinarily 0.1 mg. is the minimum amount necessary; however, single crystals smaller than 1 microgram may be identified. Tables listing the three most intense powder diffraction lines and the innermost line make possible the identification of 28 hydrazones. This method has the advantage of analyzing mixtures of carbonyl compounds which would be difficult by existing methods. The qualitative micromethod is converted to a quantitative method by accurate measurement of the line densities of known mixtures of the aldehyde derivative with sodium fluoride as an internal standard. Standard intensity ratios are given for a number of the aldehyde derivatives.

growth of the {100} form of the platelets. The angles between the faces parallel to the c axis were measured as the angles through which the needle-shaped crystal had to be rotated to bring successive faces into position to reflect a beam of light into a telescope placed on the Weissenberg camera. A cleavage plane making an angle of 52° with the c axis in the ac plane was observed.

Experimental X-Ray Data. Rotation and Weissenberg patterns for zero, first, and second layer lines were made around the b and c axes from which b_0 and c_0 unit-cell dimensions are directly measured, while a_0 is calculated from appropriate interferences on the zero layer line pattern from rotation around the b and c axes. No systematic extinctions are observed for zero, first, or second layer lines.

Since greater precision could be obtained with the powder camera of 7.0-cm. radius, patterns were made both on Agfa nonscreen film and Eastman Type A fine grain film; the latter pattern was then microphotometered with the Leeds & Northrup recording instrument.

The construction of the reciprocal lattice projection of a triclinic unit cell cannot be made with a simple drawing as can be done with orthogonal unit cells. From the projection of the XY plane the lines may be indexed as arising from $hk0$ planes; the XZ plane projection indexes the lines $h0l$ and the YZ projection indexes the lines as from $0kl$ planes. These reciprocal lattice projections have been constructed from data obtained from the Weissenberg patterns as follows:

Consider first the lines from the planes $hk0$. The interferences from the zero layer line photograph about the c axis were tabulated in order of decreasing "d" values. The lines on the powder pattern will occur in this same order and may be rapidly indexed until the planes begin to overlap with the planes having indexes $0kl$, $h0l$, or hkl. The intensity measurements serve as a check. The Weissenberg pattern of the zero layer line about the b axis was used in a similar manner to construct the $h0l$ lattice layer. This procedure cannot be followed in indexing the YZ plane ($0kl$), since no Weissenberg pattern was obtained with the crystal rotated about the a axis. However, it was observed that the $0\bar{2}1$ spot on the first layer line Weissenberg pattern of the crystal rotated about the c axis was very intense. Calculation of the "d" value of this spot on the Weissenberg film leads to a value of 3.08 Å. This calculation was made using the equation

$$\cos 2\theta = \cos \phi \cos \mu$$

where θ is the Bragg angle, ϕ is equal to $(x/r)360/2\pi$ (where x is the measured distance of the spot from the center of the film, and r is the radius of the camera), and $\mu = \tan^{-1} q/r$ (where q is the distance from the zero to the first layer line). Knowing that line 13 on the powder pattern is the $0\bar{2}1$ line, it is possible to draw the reciprocal lattice projection of the $0kl$ plane.

From the reciprocal lattice projections of the powder pattern together with single crystal data from Laue, rotation, oscillation, and Weissenberg patterns it is possible to describe the unit cell with fair accuracy. We may then say that the unit cell of formaldehyde–2,4-dinitrophenylhydrazone is triclinic:

$a_0 = 10.00 \pm 0.03$ Å. $\quad \alpha = 94°$
$b_0 = 10.41 \pm 0.05$ $\quad \beta = 95°$
$c_0 = 4.23 \pm 0.03$ $\quad \gamma = 87°$

Since there were no systematic extinctions observed, the space group must be P1 or P$\bar{1}$. From the measured density of the crystal, the number of molecules per unit cell is 2.08 according to the formula

$$n = \rho V/1.65 M$$

where ρ is the density, V the volume of the unit cell in cubic Ångströms, and M is the molecular weight. This indicates two molecules per unit cell. The space group P1 has one equivalent position per unit cell, while space group P$\bar{1}$ has two. Since the formaldehyde–2,4-dinitrophenylhydrazone molecule must be asymmetric we may ascribe the probable space group P$\bar{1}$ to this crystal.

POLYMORPHISM OF 2,4-DINITROPHENYLHYDRAZONES

Proceeding in similar fashion 11 other aldehyde and 2 ketone derivatives have been analyzed as summarized in Table I. Only the formaldehyde and crotonaldehyde compounds have only one form; all others are di- or trimorphic. Analyses have indicated that these variations are not the result of impurities. In all cases the original aldehydes and ketones were purified so that any sample had a boiling point range of less than 1°. The powder diffraction spacings which are listed in Tables II and III are highly characteristic and reproducible for each compound. Both

Table I. Crystallographic Data of 2,4-Dinitrophenylhydrazones

Compound	Color	Habit	Solvent	M.P., °C.	System	a_0	b_0	c_0	Axial Angle	Density	n	S.G.
Formaldehyde	Yellow	Tabular (010)	Isobutanol	166	Triclinic	10.00	10.41	4.23	$\alpha = 94°$ $\beta = 95°$ $\gamma = 87°$	1.592	2	P$\bar{1}$
Acetaldehyde I	Yellow	Tabular (001)	n-Propanol	166	Tetragonal	7.15	7.15	18.69	.....	1.541	4	$P4_2/n$
Acetaldehyde II	Orange	Acicular[a]	n-Propanol	165	Orthorhombic	5.06	10.6	17.3	.....	1.51	4	$P2_12_12$
Propionaldehyde I	Red	Acicular[a]	Xylene	150	Orthorhombic	5.34	11.44	17.35	.....	...	4	$C22_12$
Propionaldehyde II	Orange	Acicular	Ethanol	148	.....	...	...	...	.....	...	..	....
n-Butyraldehyde I	Yellow	Acicular[a]	Methanol	123	Orthorhombic	4.90	17.9	54.0	.....	1.3	..	....
n-Butyraldehyde II	Orange	Acicular[a]	Methanol	122	Orthorhombic	7.0	25.0	25.8	.....	1.3	..	....
n-Butyraldehyde III	Amber	Tabular	Methanol	122	Orthorhombic	7.55	...	25.3	.....	...	..	....
Crotonaldehyde	Red	Acicular[a]	Benzene	190	Orthorhombic	4.63	13.05	18.30	.....	1.43	4	$P2_12_12$
Furfuraldehyde I	Red (deep)	Prisms	Acetone	223	Monoclinic	7.63	13.23	13.07	$\beta = 99°$	...	4	$P2/m$
Furfuraldehyde II	Red (light)	Pyramids	Acetone	218	Monoclinic	?	?	28.3	.....	...	..	....
Furfuraldehyde III	Yellow	Acicular[a]	Ethylene dichloride	199	Monoclinic	3.63	11.7	7.7	$\beta = 95°$	...	1	$P\bar{2}/m$
Dimethyl ketone I	Yellow	Tabular	Ethanol	126	Triclinic	7.12	8.04	9.91	$\alpha = 66°$ $\beta = 86°$ $\gamma = 102°$	1.42	2	P$\bar{1}$
Dimethyl ketone II	Yellow	Acicular	Ethyl ether	114	Orthorhombic	5.22	11.0	22.6	.....	1.4	4	$P2_12_12$

[a] Elongation parallel to a axis.

temperature and solvent are factors in crystallization of a particular type. In two instances two kinds of crystals have appeared together but could be easily separated mechanically. An interesting general property observed is that the acicular crystals were all elongated parallel to the shortest unit cell dimension, and the tabular crystal had planes of flattening which were normal to the longest unit cell dimension.

Several of these modifications have not been reported in the literature as such—namely, acetaldehyde II, propionaldehyde II, butyraldehyde II and III, furfuraldehyde I, and dimethyl ketone II. On the other hand the reported acetaldehyde derivation with stable form from sublimation (*6*) could not be reproduced with as careful repetition of conditions as possible.

QUALITATIVE ANALYSIS WITH MICRO QUANTITIES FROM POWDER DIFFRACTION PATTERNS

Table II, which gives interplanar spacing "d" values and intensities of the three or four most intense lines from the powder x-ray diffraction patterns of the aldehyde–2,4-dinitrophenylhydrazones whose unit cell dimensions and space groups have been determined and listed in Table I, and Table III, of those values for ketone derivatives, provide a system of qualitative analysis for aldehydes and ketones. The amount of 2,4-dinitrophenylhydrazine derivative necessary for an x-ray powder pattern is about 0.1 to 0.5 mg.; however, satisfactory single crystal patterns have been taken of crystals smaller than 10^{-6} cc. (1 microgram). Fortunately, the required degree of purity is not nearly so great as that required for melting point determinations and the impurities themselves may be determined if present in quantities greater than 5%.

Apparatus. The apparatus necessary for preparing the derivative in micro quantities may be easily assembled from glass tubing and commercial semimicro equipment. The essential equipment consists of eye droppers with the ends drawn into short capillary tubes; small centrifuge tubes with constricted end for collecting the precipitate into a compact small space; small centrifuge and x-ray powder cameras.

For the x-ray patterns the powder samples were held in cellulose acetate tubes, prepared in a manner similar to that described by Fricke, Lohrmann, and Schroeder (*9*). For this qualitative analysis of very small amounts, the cellulose acetate tubes prepared on No. 24 Chromel wire have been found more suitable than those on copper wire, since in small sizes Chromel wires are more easily handled than copper wires. After stretching the wire coated with the cellulose acetate, the tubes have an internal diameter of approximately 0.35 mm.

The powder camera was designed for recording diffraction patterns of organic materials. The radius from sample to film is 7.00 ± 0.01 cm. Minimum exposure time is gained by placing the back-defining pinhole of 0.254- or 0.635-mm. (0.010- or 0.025-inch) diameter on the circumference of the camera and a guarded pinhole of 0.635 or 1.02 mm. (0.025 or 0.40 inch) in place 1.25 cm. (0.5 inch) from the sample. With these relatively large front pinholes, exposure times could be shortened without appreciably losing definition through line broadening from angles greater than 20° from the central beam. A conically machined collimating tube holding the camera to the Machlett-type diffraction tube assures positive alignment of the x-ray beam. A small beam trap and central mounting of the specimen rotated or oscillated by a reversing motor permit the registration of spacings of less than 40 Å. and measurement on both sides of the central beam.

Technique of Qualitative Analysis. The sample for such an exposure as that of formaldehyde–2,4-dinitrophenylhydrazone was prepared by adding approximately 1 drop of a 40% formaldehyde solution with an eye dropper to a 0.3 ml. of a methyl alcohol–hydrochloric acid solution of 2,4-dinitrophenylhydrazine in one of the small centrifuge tubes. This solution of 2,4-dinitrophenylhydrazine was prepared by dissolving 1 gram of 2,4-dinitrophenylhydrazine in 25 ml. of methyl alcohol containing approximately 2 equivalents of hydrochloric acid gas per liter of alcohol. Such a solution has been found relatively more stable for high concentrations of 2,4-dinitrophenylhydrazine than ethyl alcohol–sulfuric acid solutions. After allowing the solution of the formaldehyde derivative to stand 0.5 hour the tube was centrifuged, the liquid decanted, and the precipitate washed twice with 95% ethyl alcohol, then centrifuged and decanted again. A bit of filter paper was used to remove most of the wash liquid, then the remainder was removed by attaching the centrifuge tube to a vacuum pump for 15 minutes.

A portion of the dry sample in the constricted end of the centrifuge tube was transferred to a cellulose acetate tube by forcing some of the sample into the open end of the cellulose acetate tube, then tamping the sample solidly into tube with another Chromel wire.

Evaluation of Powder Patterns. Since there is a possibility of contaminating the sample with unchanged 2,4-dinitrophenylhydrazine and its hydrochloride, these powder patterns were taken; also a powder pattern of the hydrochloride after "decomposition" in a vacuum for 2 hours. This decomposition is accompanied by a change in color from yellow to orange red. These together with the derivatives of formaldehyde, acetaldehyde (2 forms), propionaldehyde (2 forms), butyraldehyde (3 forms), crotonaldehyde, and furfuraldehyde (3 forms) are listed in Table II with the three most intense lines in order of their relative intensities in a manner similar to that proposed by Hanawalt, Rinn, and Frevel (*11*). Since the innermost line in all cases is usually the most characteristic, it is also tabulated. Table III similarly lists these data for 16 ketone–2,4-dinitrophenylhydrazones. The use of the tables facilitates the identification of the unknown derivative. Further confirmation of the other weaker lines in the powder pattern of the unknown may be made by reference to the tables of "d" values listed for each standard pattern which in the interest of brevity are not tabulated in this paper.

Table II. "d" Values of Most Intense Lines of 2,4-Dinitrophenylhydrazine and Its Aldehyde Derivatives in Order of Decreasing Intensity

(Relative intensity I/I_0 in parentheses)

Compound	I	II	III	IV[a]
2,4-D.P.H.	3.50 (1.0)	3.15 (0.6)	5.81 (0.6)	8.9 (0.5)
2,4-D.P.H.–HCl	3.18 (1.0)	3.54 (0.8)	2.68 (0.5)	8.0 (0.2)
2,4-D.P.H.–HCl decomposed in vacuum	3.11	3.91	4.51	7.0
Formaldehyde, yellow	3.08 (1.0)	3.62 (0.97)	3.47 (0.88)	10.3 (0.86)
Acetaldehyde I, yellow	3.21 (1.0)	9.35 (0.70)	4.65 (0.37)	9.35 (0.70)
Acetaldehyde II, orange	9.2 (1.0)	3.20 (0.92)	6.8 (0.38)	9.2 (1.0)
Propionaldehyde I, red	9.6 (1.0)	3.24 (0.70)	4.30 (0.39)	9.6 (1.0)
Propionaldehyde II, orange	11.0 (1.0)	3.30 (0.8)	4.75 (0.3)	11.0 (1.0)
n-Butyraldehyde I, yellow	14.0 (1.0)	3.77 (0.32)	3.21 (0.31)	14.0 (1.0)
n-Butyraldehyde II, orange	11.7 (1.0)	3.50 (0.38)	3.48 (0.29)	13.7 (0.16)
n-Butyraldehyde III, amber	12.6 (1.0)	3.28 (0.83)	4.33 (0.26)	12.6 (1.0)
Crotonaldehyde, red	3.22 (1.0)	3.26 (1.0)	10.9 (1.0)	10.9 (1.0)
Furfuraldehyde I, red-black	3.17 (1.0)	3.20 (0.76)	4.20 (0.58)	13.7 (0.4)
Furfuraldehyde II, red	3.24 (1.0)	5.68 (0.3)	5.36 (0.19)	11.7 (0.03)
Furfuraldehyde III, yellow	3.26 (1.0)	6.17 (0.5)	7.7 (0.34)	11.7 (0.29)

[a] Innermost line.

Table III. "d" Values of Most Intense Lines of Ketone–2,4-Dinitrophenylhydrazones in Order of Decreasing Intensity

Compound of Ketone	I	II	III	IV[a]
Dimethyl I, yellow	3.27 (1.0)	5.70 (0.62)	9.30 (0.54)	9.30 (0.54)
Dimethyl II, yellow needles	9.45 (1.0)	11.15 (0.44)	3.03 (0.20)	11.15 (0.54)
Ethyl methyl, orange	12.45 (1.0)	3.25 (0.71)	7.10 (0.54)	12.45 (1.0)
n-Propyl methyl, yellow-orange	3.61 (1.0)	12.50 (0.78)	10.64 (0.66)	12.50 (1.0)
n-Butyl methyl, orange	12.80 (1.0)	7.75 (0.72)	3.47 (0.56)	12.80 (1.0)
n-Amyl methyl, yellow-orange	13.20 (1.0)	3.47 (0.70)	7.48 (0.48)	13.20 (1.0)
Isobutyl methyl, red-orange	13.70 (1.0)	7.60 (0.78)	3.48 (0.42)	13.70 (1.0)
Diethyl, red-orange	12.60 (1.0)	7.10 (0.76)	3.54 (0.37)	12.6 (1.0)
Di-*n*-propyl, yellow	14.9 (1.0)	3.44 (0.56)	7.80 (0.30)	14.9 (1.0)
Diisopropyl, red-orange	13.7 (1.0)	6.90 (0.78)	3.40 (0.62)	13.7 (1.0)
Diisobutyl, orange	11.20 (1.0)	3.72 (0.63)	4.45 (0.42)	11.2 (1.0)
Pinacalone, yellow	14.0 (1.0)	3.16 (0.52)	7.42 (0.50)	14.0 (1.0)
Cyclopentanone, orange	11.1 (1.0)	3.23 (0.58)	5.80 (0.32)	11.1 (1.0)
Benzyl methyl, beet red	3.31 (1.0)	10.5 (0.82)	6.15 (0.78)	10.5 (0.82)
p-Cl-acetophenone, scarlet	3.30 (1.0)	6.10 (0.60)	5.35 (0.42)	12.3 (0.32)
Benzophenone, red-orange	18.2 (1.0)	4.37 (0.80)	8.80 (0.75)	18.2 (1.0)

[a] Innermost line.

Table IV. Quantitative Data on Aldehyde–2,4-Dinitrophenylhydrazones–Sodium Fluoride Patterns

Compound	Weight Ratio, D.P.H./NaF	Intensities NaF (200)	Intensities NaF (220)	Intensities D.P.H.	Intensity Ratios D.P.H./NaF (200)	Intensity Ratios D.P.H./NaF (220)
Formaldehyde	2.54	0.566	0.273	0.239	0.42	0.87
		0.607	0.295	0.254	0.43	0.86
	1.23	0.950	0.490	0.185	0.195	0.378
		0.968	0.472	0.207	0.214	0.440
	0.915	0.640	0.303	0.090	0.140	0.296
		0.680	0.328	0.100	0.147	0.305
	1.00 (av.)	...	...	...	0.165	0.338
Acetaldehyde I (yellow)	17.70	0.876	0.440	0.549	0.63	1.25
		0.855	0.428	0.506	0.59	1.18
	3.19	0.590	0.281	0.685	1.16	2.44
		0.551	0.274	0.579	1.05	2.11
	2.66	0.992	0.500	0.807	1.81	1.61
		1.11	0.521	0.910	1.82	1.74
	1.00 (av.)	...	...	...	0.345	0.695
Propionaldehyde I (red)	2.33	1.22	0.77	0.72	0.59	0.935
		1.19	0.70	0.72	0.60	1.03
	2.37	1.06	0.65	0.75	0.71	1.15
		1.06	0.67	0.68	0.64	1.01
	1.00 (av.)	...	...	...	0.270	0.438
Butyraldehyde I (yellow)	1.95	0.400	0.239	0.51	1.127	2.13
		0.370	0.203	0.45	1.22	2.21
	1.63	0.845	0.417	0.790	0.935	1.89
		0.727	0.398	0.770	1.06	1.93
	1.00 (av.)	...	...	...	0.597	1.14
Crotonaldehyde	1.90	1.05	0.505	0.458	0.436	0.907
		1.06	0.534	0.447	0.420	0.84
	1.15	1.32	0.715	0.370	0.280	0.519
		1.25	0.630	0.367	0.293	0.580
	1.00 (av.)	...	...	...	0.235	0.465
Furfuraldehyde I (red)	2.08	1.20	0.610	0.540	0.45	0.89
		1.19	0.622	0.470	0.393	0.755
	1.00 (av.)	...	...	...	0.202	0.395
2,4-D.P.H. (uncombined)	1.57	0.885	1.75	0.570	0.325	0.645
		0.970	1.87	0.638	0.340	0.657
	1.60	0.955	2.07	0.635	0.317	0.665
		0.910	1.60	0.560	0.350	0.615
	1.00 (av.)	...	...	...	0.209	0.408

QUANTITATIVE X-RAY ANALYSIS

The quantitative analysis of pure aldehydes by precipitation with 2,4-dinitrophenylhydrazine has been studied carefully by Iddles and Jackson (*12*), who reported yields greater than 95% for simple aliphatic aldehydes. Their method, using standard quantitative procedures, is accurate when the aldehyde may be obtained relatively free of other carbonyl compounds. The use of accurate density measurements of x-ray powder patterns of samples containing some internal standard makes possible quantitative analysis of samples of mixed aldehydes and ketones.

Quantitative analysis by the x-ray powder diffraction method has been reported for the analysis of mineral and metallurgical samples (*10*) using sodium chloride as an internal standard. These articles have dealt rather carefully with the theory of line intensities but lack in the presentation of satisfactory laboratory procedures. These articles report an accuracy of 5% in the determination. This is probably a good estimate for most crystalline compounds, but under favorable circumstances the accuracy may be greater.

The necessary steps for obtaining a good quantitative estimation of a mixture of two known compounds are then:

1. Calibration of the film used.
2. Preparation of a known mixture of the compounds in question with some definite amount of an internal standard such as sodium fluoride. The sample must be prepared in a finely divided state and thoroughly mixed, either by rapid evaporation or fine grinding, using some inert liquid as a dispersing agent.
3. Measurement of the relative line densities of the internal standard lines and the lines of the sample under consideration.
4. Correction of the relative line densities to give line intensities by using the film calibration curve.
5. Calculation of the ratio of line intensities of the internal standard and sample in question for a 1 to 1 mixture.
6. Preparation of another sample containing an unknown amount of the constituent in question with a known percentage of the internal standard.
7. Determination of the relative line densities from the powder pattern.
8. Correction of density to give intensity of the lines.
9. Calculation of the ratio of line intensities of unknown to internal standard. Multiplication of this factor by the ratio determined in step 5 will give the ratio weight of unknown to internal standard. Knowing the amount of internal standard in the sample (step 6) subjected to the x-ray examination, it is possible to calculate the amount of constituent in question present in the original sample.

When a film is used with a linear response to the x-rays, steps 4 and 7 may be omitted.

Quantitative X-Ray Analysis of Aldehydes. The quantitative analysis of aldehydes was accomplished in this manner. Sodium fluoride was chosen as the internal standard, since it could be obtained in large quantities having a particle size small enough to give smooth sharp diffraction lines as well as produce a relatively simple powder pattern with a minimum of absorption of the x-ray beam.

The samples were prepared by mixing weighed quantities of the aldehyde–2,4-dinitrophenylhydrazone with sodium fluoride. These were ground together carefully in an agate mortar, filled in a 0.35-mm. diameter cellulose acetate tube, and exposed to the x-ray beam in the 7.00-cm. powder camera for 2.5 to 3 hours using Eastman No-Screen film. The motor on the camera was set to rotate through 360° to smooth out any spots on the diffraction lines. The patterns were microphotometered with the Leeds & Northrup recording microphotometer.

Table IV lists the intensity data from the series of aldehyde–2,4-dinitrophenylhydrazone–sodium fluoride patterns. Two different standard lines (200) and (220) are chosen for sodium fluoride and the strongest line, usually corresponding to a "d" spacing near 3.20 Å., for the hydrazones. The two sets of readings for each pattern were obtained from the two sides of the film upon which the powder pattern was registered.

The 1 to 1 ratios of line intensities in the table were calculated by dividing the ratio of intensities of the aldehyde 2,4-dinitrophenylhydrazone to sodium fluoride lines (columns VI and VII) by the weight of the constituents of the sample (column II). These 1 to 1 ratios may now be used to calculate the weight ratio of unknown to sodium fluoride in any given sample, after qualitative identification of the aldehyde.

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Determination of Methyl Chloride in Air

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A rapid and convenient method has been developed for determining low concentrations of methyl chloride in air for safety control purposes. The method consists of passing a measured volume of air through an electric spark between carbon electrodes and absorbing the products of combustion in sodium arsenite solution. The solution is slightly acidified with nitric acid, and silver nitrate is added to develop silver chloride turbidity. The amount of chloride present is determined by comparing the turbidity of the unknown with that of standards similarly prepared. From analyses of synthetic blends in concentration ranges of 50 to 1800 p.p.m. the probable error of a single determination has been calculated to be ±10%.

METHYL CHLORIDE is used in the manufacture of rubber and in other industrial chemical processes, and various amounts inevitably escape at times from the units where it is in use. Because of its toxicity, its presence in the atmosphere in excessive concentrations constitutes a hazard to workers in the area. A method was desired which would determine the amount of methyl chloride in air, so that, if allowable tolerances were exceeded, appropriate precautions might be taken. It was required that the method provide satisfactory accuracy for safety control purposes, be explosionproof for use in areas containing gaseous hydrocarbons, require short elapsed time for a determination, be specific for chlorine compounds, and be portable, so that determinations might be carried out in any designated area of the plant.

Various methods have been described for the determination of low concentrations of methyl chloride and other organic halides in air (*2–4*). Although a high degree of accuracy has been reported for these methods, none seemed to provide the required rapidity, adaptability, or freedom from the interference of such substances as organic sulfur compounds. A method which meets the specifications reasonably well has been developed and is reported herein with experimental results obtained by the method in analyses of known methyl chloride–air blends.

APPARATUS[1]

Diagrams of the reactor tube and of the complete assembly for analysis of methyl chloride are shown in Figures 1 and 2.

The reaction chamber is made of glass, and surgical rubber tubing is used throughout to provide airtight connections. The purpose of the copper collar (*B*, Figure 1) is to prevent loss of air sample by diffusion through the carbon electrodes and to afford electrical contact with the carbon electrodes. The glass plug, *E*, serves to make the apparatus airtight and to aid in spacing the electrode gap. The flame arrester, *G*, which consists of a 2.5-cm. (1-inch) length roll of 100-mesh copper gauze inserted into a glass tube, prevents explosion of hydrocarbon mixtures which might be drawn into chamber *A*, Figure 2, which should be of known volume. A vessel of 250- to 300-ml. capacity should provide a sample large enough to give satisfactory accuracy for most measurements made for safety control purposes; however, for concentrations less than 200 p.p.m., greater accuracy may be provided by use of a larger volume of sample. The chamber is fitted with a three-way stopcock, so that the sample may be drawn into it directly from the area to be tested and later passed through the reactor.

The electrodes are made of 0.25-inch diameter spectrographic carbon rods. A supply of hollow electrodes (*C*, Figure 1) is prepared by the use of 0.125- and 0.063-inch metal drills. The absorber, *D*, is fitted with a fritted-glass disperser to ensure complete scrubbing of the combustion products. The fritted-glass disk should be porous enough so that a pressure of 4 to 6 cm. of mercury is adequate for passage of the sample through the sodium arsenite solution at the desired rate, in order to minimize the possibility of leakage. Experimentation has shown that one absorber is sufficient to ensure complete removal of chlorine and its compounds from the combustion products.

The complete assembly with such accessory parts as Nessler tubes, standard solutions, and buret should weigh less than 40 pounds when built into a compact portable unit. The electrical parts may be installed in this unit in a way which eliminates the explosion hazard of an exposed electric spark. As an alternative method of determining chlorides, a nephelometer may be built into the unit, and undoubtedly will provide greater accuracy of measurement than that provided by visual comparison of the unknown with turbidity standards. Since the apparatus can be used wherever 110-volt alternating current outlet is available, practically any point in the plant is accessible for a determination.

The apparatus must be checked to make sure that all connections are airtight, and any worn rubber connections replaced. Any leakage will, of course, reduce the accuracy of the determination.

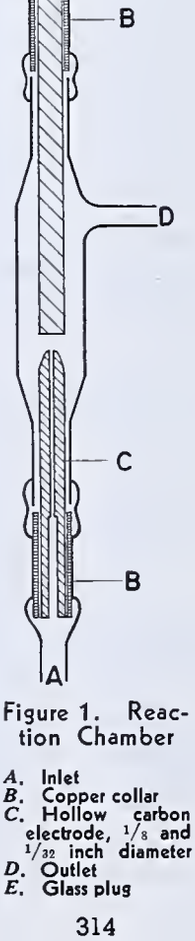

Figure 1. Reaction Chamber

A. Inlet
B. Copper collar
C. Hollow carbon electrode, 1/8 and 1/32 inch diameter
D. Outlet
E. Glass plug

REAGENTS

Sodium arsenite, approximately 0.1 *N*. Dissolve 4 grams of C.P. sodium hydroxide, then 4.9 grams of C.P. arsenic trioxide in 50 ml. of water. Make up to a volume of 1 liter with distilled water.

Standard chloride, 1 ml. containing 0.1 mg. of chloride. Dissolve 1.648 grams of fused C.P. sodium chloride in 1 liter of distilled water. Dilute 100-ml. aliquot of this solution to 1 liter to prepare a solution containing 0.1 mg. of chloride per ml.

Silver nitrate, approximately 1 *N*. Dissolve 17 grams of C.P. silver nitrate in 100 ml. of distilled water.

Nitric acid, concentrated C.P. stock reagent.

Phenolphthalein indicator.

The use of chloride-free reagents throughout is emphasized.

PROCEDURE

The sample to be analyzed is drawn by mercury displacement into chamber *A*. Before closing the stopcock to the outside after drawing in the sample, the stopcock between *A* and *B* is closed, so that the sample in *A* will be at atmospheric pressure.

About 15 ml. of 0.1 *N* sodium arsenite solution are added to absorber *D*. Sodium arsenite is used as the absorption medium, so that any chlorine or oxychlorine compounds that may be formed in the spark will be reduced to chloride.

The spark is started by connecting the primary of transformer *F* to a 110-volt alternating current supply. It is important that the electrodes be properly spaced and in good condition, so that an even spark will be obtained. Electrodes too greatly separated do not spark evenly, while those too close together tend to generate too much heat. This spacing must be determined by trial. Occasionally the electrodes become badly pitted and must be removed and reshaped. The reshaping can easily be done with a pencil sharpener and a knife.

The air sample in *A* is passed through the spark in the reactor and absorber *D* by raising the mercury reservoir, *B*, opening the three-way stopcock to connect *A* and *C*, and opening the stopcock between *A* and *B* enough to permit passage of a 275-ml. sample in 7 to 8 minutes. With experience the operator can learn to adjust the flow quickly to the desired rate of 30 to 40 ml. per minute. When all the air sample has been forced through the reactor, the one-way stopcock below *B* is closed immediately and the electrode spark discontinued. One hundred milliliters of chloride-free air should be used to flush out the reactor after each determination, since there is some retention of reaction prod-

[1] Patent applied for.

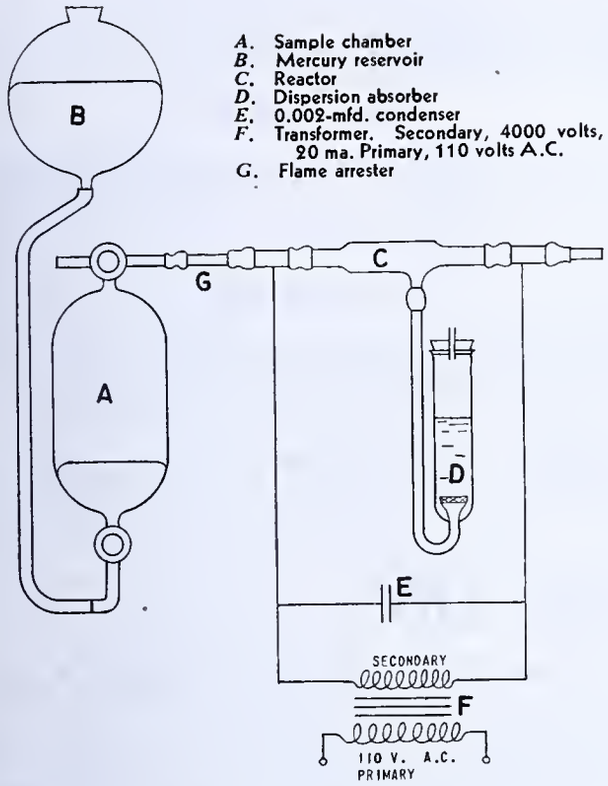

Figure 2. Complete Assembly

cts in the reactor. A small container of chloride-free air may e used for this purpose. Care must be taken to prevent drawing odium arsenite solution into the reactor or forcing mercury into ; otherwise the reactor will have to be taken apart and cleaned efore it can be used again.

After passage and combustion of the air sample, the sodium rsenite solution from absorber *D* is poured into a 50-ml. beaker nd the absorber rinsed with three 5-ml. portions of distilled vater which is also added to the beaker. A drop of phenol-hthalein indicator is added to the solution, which is then acidi-ied with an excess of 3 drops of concentrated nitric acid. The cidified solution is poured into a 50-ml. Nessler tube, made up o volume with distilled water, and 3 drops of 1 *N* silver nitrate re added. The Nessler tube is stoppered and inverted several imes to mix the solution thoroughly. For concentrations of nethyl chloride greater than 1000 p.p.m., an aliquot part of the bsorber solution should be taken for the chloride determination, o that the turbidity will not be too great to prevent satisfactory omparison with the standards.

Turbidity standards are prepared for comparison with the un-nown by adding measured amounts of standard chloride solu-ion to Nessler tubes, each containing 15 ml. of 0.1 *N* sodium arse-ite neutralized and acidified with concentrated nitric acid. The tandards are made up to volume with distilled water and 3 drops f 1 *N* silver nitrate are added. The amounts of chloride are hosen so that the unknown is matched or closely bracketed by he standard turbidity tubes. The standards are prepared at the ame time the unknown sample turbidity tube is being prepared. With practice the operator can learn to complete an analysis in 30 minutes.

CALCULATION

The concentration of methyl chloride in the sample is calculated rom the following formula:

$$\text{P.p.m. of methyl chloride} = \frac{N \times 1{,}000{,}000}{13.67 \times V}$$

N is the milliliters of standard chloride equivalent to the test ample in turbidity. *V* is the volume in milliliters of sample ested. The factor is obtained as follows:

The weight of combined chlorine in 1 ml. of methyl chloride at tandard pressure and 27° C. is 1.44 mg. Since each milliliter of tandard chloride solution contains 0.1 mg. of chloride, 1 ml. of methyl chloride gas is equivalent to 14.4 ml. of standard chloride olution. On multiplying 0.95, a correction factor to compensate for systematic error, by 14.4, the value 13.67 is obtained.

If an aliquot part of the absorber solution has been used to develop the turbidity of the unknown, the appropriate correction is made in calculating the concentration of methyl chloride.

EXPERIMENTAL

To evaluate the present method, synthetic blends for analysis were prepared by introducing methyl chloride, measured at room temperature over mercury in a buret, into a partially evacuated carboy. Analysis by combustion showed the methyl chloride used to be 96% pure; this purity value was taken into account in preparing the blends.

A carboy of 11.5-liter capacity was used to prepare blends containing more than 200 p.p.m. and a carboy of 19-liter capacity for those containing less. After admitting chloride-free air to atmospheric pressure, a 275-ml. sample was withdrawn into chamber *A* for analysis. Two withdrawals of 275 ml. each were made in some cases for samples containing less than 200 p.p.m. of methyl chloride. It was found that the relative error of a determination was less using a 550-ml. sample than using a 275-ml. sample of the same concentration. For safety control purposes the use of larger volumes of sample than 275 ml. will probably be unnecessary.

Distinction between a series of turbidities produced by 0.1-ml. increments of the standard chloride up to 1.5 ml. can be made by direct observation. Distinction between 0.2-ml. increments can be made up to 4.0 ml. of standard chloride, although the distinction is less easily made as the amount of turbidity is increased. These distinctions can best be made by examining the Nessler tubes vertically and against a dark background. As little as 0.005 mg. of chloride can readily be detected when compared with a blank. When a volume of 275 ml. of sample is analyzed, 0.1

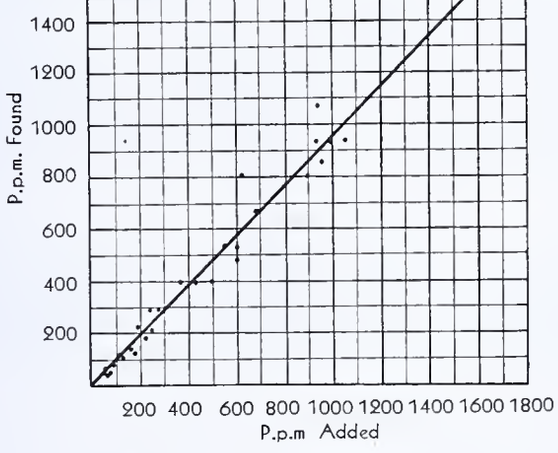

Figure 3. Methyl Chloride Concentration in Air Blends vs. Concentration as Found by Analysts

ml. of standard chloride corresponds to a concentration of approximately 26 p.p.m. of methyl chloride. Although measurements of turbidity by direct visual examination may be satisfactory for control purposes, the use of a nephelometer would undoubtedly improve the accuracy of these measurements (*1*).

RESULTS

The results of several determinations made on synthetic blends of high-purity methyl chloride in air containing 50 to 1800 p.p.m. of methyl chloride are shown graphically in Figure 3. Inspection of the data in which "p.p.m. added" values were compared with corresponding "p.p.m. found" values suggested that a systematic error was operative in the method. By application of the method of least squares to the data the slope of the curve of Figure 3 was calculated to be 0.95. This indicates that a detection of 0.95

of the concentration of methyl chloride present in a given sample of air may be expected by the method. Therefore this correction factor is included in the calculation to compensate for the systematic error. It is believed that the systematic error is attributable to the retention in the reactor of combusion products that are not completely removed by rinsing with chloride-free air.

OTHER APPLICATIONS

It is contemplated that the method described in this article will prove useful in the detection and determination of traces of various organic halogen compounds such as refrigerants in air. The method may, with proper modifications, be applied to the determination of traces of methyl chloride in hydrocarbon gases. Although a few determinations of methyl chloride in hydrocarbons containing organic sulfur compounds have been made by this method, no controlled work to evaluate its accuracy has been done. With complete combustion no interference resulted from the presence of organic sulfur compounds.

ACKNOWLEDGMENT

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Field Test for Surface DDT

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A simple field test for surface DDT is presented by which a determination can be completed in 2 to 3 minutes. Conditions which may be encountered in using the test are indicated.

THE application by spray of DDT in solution or in an emulsion, so as to leave a surface residue, presents numerous opportunities for using a fast simple field test to detect the presence of this substance on the surface. By means of such a test the many factors which may affect the concentration of DDT left on a surface after spraying may be studied and perhaps controlled, thus bringing about the development of more efficient and economical methods of application.

The present communication presents a test based upon a development of the xanthydrol–potassium hydroxide–pyridine reaction for DDT (*2*). It is simple, sensitive, rapid, and may be used in the field.

PRINCIPLE

DDT is removed from a surface by scrubbing with a cotton swab impregnated with mineral oil. The swab is then subjected to a modified xanthydrol–potassium hydroxide–pyridine reaction. In the presence of DDT a red color is secured.

REAGENTS AND APPARATUS

Only two reagents are required: a 0.4% solution of xanthydrol in pure anhydrous pyridine, and potassium hydroxide pellets, U.S.P. (either Merck or Baker). Some lots of C.P. pyridine are sufficiently anhydrous for use without purification, but most samples contain water. This may be easily removed by allowing the compound to stand in contact with stick sodium hydroxide for several days and then distilling. The xanthydrol-pyridine solution must be kept in a glass-stoppered bottle, and may be used for several days without deterioration. The hydroxide should be kept in a tightly capped container when not in use, in order that the pellets may absorb as little water as possible.

The apparatus consists of a supply of clean dry 17 × 150 mm. test tubes, a small alcohol lamp, a test tube holder, a 2-ml. pipet, and a number of oil-impregnated swabs. The swabs are prepared by wrapping a bit of cotton tightly around the end of an applicator stick, dipping it in clear liquid petrolatum, and then squeezing out the excess against the side of the container.

The reagents and apparatus may be packed into a suitable box or case for ease in carrying. The set used by the authors consists of a 72-tube wire test-tube rack such as is used for Wassermann tests. Wires were cut out to provide space for the reagent bottles and the lamp, as well as for a large tube containing swabs. This arrangement provides a rack for a dozen or more test tubes. The pipet may be carried in one of the tubes or may be secured to the rear, together with the test-tube holder, by means of clips.

PROCEDURE

An area of about 12 square inches (3 × 4 inches) of the surface supposed to contain DDT is thoroughly scrubbed with the oil impregnated swab. A test tube is then inserted into the hydroxide bottle, and two pellets are picked up on the lip and allowed to slide to the bottom of the tube. The bottle is immediately recapped to prevent absorption of moisture. Two milliliters of 0.4% xanthydrol in pyridine are then added to the tube by means of the pipet. Using the holder, the tube is kept at a gentle boil over the alcohol lamp by moving it in and out of the flame. When the contents turn green, the swab is inserted, and the tube boiled for a few seconds longer. If DDT is present, the color changes to red. If no DDT is present, the green fades quickly to yellow. The entire procedure takes from 2 to 3 minutes.

EXPERIMENTAL

A large number of different surfaces known not to contain DDT, such as brick, cement, stone, tile, glass, paper, plaster, screen, wood, paint, metal, plastics, canvas, fruit, foliage, grass, etc., were swabbed and tested. No false positives were secured.

DDT in ether solution in concentrations varying by increments of 5 micrograms was measured directly onto a series of swabs. The ether was allowed to evaporate at room temperature and the swabs were subjected to the test. Twenty-five micrograms of DDT were detectable by comparison with a blank while 75 micrograms gave a very distinct pink color.

DDT in 5% kerosene solution and 5% aqueous triton-xylene emulsion was applied to 3 × 4 inch (12 square inches) surfaces of commonly encountered materials in concentration varying by increments as follows: 1 mg. per square foot from 1 to 10 mg. per square foot, 5 mg. per square foot from 10 to 100 mg. per square foot, 25 mg. per square foot from 100 to 1000 mg. per square foot, 50 mg. per square foot from 1000 to 2000 mg. per square foot. Except in concentrations up to 10 mg. per square foot where 1 to 10 dilutions with kerosene and water were made the solution or emulsion was applied without dilution. Application was made directly to the surface by means of micropipets spotting the entire surface so as to obtain a uniform distribution of small drops in the lower concentrations, and a film of liquid in the higher concentrations. This was done to approximate the action of a spray, and at the same time accurately control the amount applied, as accurate control of the amount applied with a spray would have been impossible. After exposure for 48 hours at room conditions, the entire surface was scrubbed with a swab and tested for DDT. A positive test was considered as one which matched the color secured with a swab containing 75 micrograms of DDT. The minimum concentrations at which the test was positive are given in Table I.

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Table I. Minimum Concentration of DDT in Positive Test

(g. per square foot, as applied, at which positive tests were secured on rious surfaces after application of DDT as a 5% kerosene solution and a 5% triton-xylene-water emulsion.)

Material	Kerosene Solution	Triton Emulsion
nt canvas, untreated	1500	1500
lotex building board, unpainted	1000	1000
bber matting, ribbed	750	50
ft pine board, unpainted	100	75
lotex building board, oil paint surface	250	25
ster, oil paint surface	200	25
ft pine board, oil paint surface	200	15
ster, fresh, smooth surface, unpainted	15	25
eet metal, galvanized surface	5	5
ss, plate	3	3

DISCUSSION

The xanthydrol–potassium hydroxide–pyridine reaction is not ecific for 2,2-bis-(*p*-chlorophenyl)-1,1,1-trichoroethane but is ven by a number of related compounds containing aliphatic logen (*1*, *2*). With due caution in using the test, however, this ctor should not seriously affect its value, as the presence of ch compounds is usually known.

There are obviously many factors which may influence the ncentration of DDT upon a surface after its application in lution or emulsion. It is the purpose of this communication to esent a simple method which may aid in the study of these ctors, rather than to give detailed consideration to the factors emselves. Such things as the porosity of the surface to the solvent used, the presence or absence of moisture on the surface or in the material, the size of the droplets deposited by the spray, and even the temperature and relative humidity at the time the spray was applied may be important in determining the amount of DDT left on a surface. For this reason a statistical evaluation of the application of the test is impossible at the present time, and the data are presented only to convey an idea as to what may be expected in using the method. On the same basis, it is impossible to control all the conditions of experimental work, so that the figures are more a representation of order or magnitude than exact data. One rather definite conclusion can be drawn from the experiments, however. If conditions are such that more than 0.075 mg. of DDT can be picked up on the swab by scrubbing the surface, a positive test will be secured.

The experimental data presented deal with the surfaces of building materials. These materials were used because of their availability and because they suited the conditions of the experiment. Application of the test to fruit, grass, vegetation, and foliage leads the authors to believe that the conditions encountered in testing building materials apply to these surfaces also. In general, fruit and green foliage, being relatively nonporous, will retain a high concentration of DDT on the surface, while dry vegetation, absorbing more of the solution or emulsion, will have less of a surface residue.

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Colorimetric Determination of Fatty Acids and Esters

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A rapid colorimetric method for determination of fatty acids and esters is based on the formation of hydroxamic acid from fatty esters by the use of hydroxylamine hydrochloride in alkaline media. On the addition of an acidified solution of alcoholic ferric perchlorate, a stable red colored complex of ferric hydroxamate is formed, proportionate in intensity to the esters present. Fatty acids are first quantitatively methylated in an anhydrous ether solution with diazomethane or thionyl chloride. The method has been applied to the quantitative determination of oil on tin plate The results are in good agreement with a gravimetric determination.

EXISTING methods for the estimation of fatty acids and their esters are time-consuming and tedious and often are inaccurate when interfering substances are present. This investigation was undertaken to develop a rapid and accurate method for the determination of small amounts of palm oil, cottonseed oil, dibutyl sebacate, and lanolin applied to tin plate and other sheet metal surfaces for rust prevention, lubrication, or other purposes in subsequent manufacturing operations.

EQUIPMENT USED

Coleman Model 11 spectrophotometer and Cenco-Sheard-anford filter photelometer.

EXPERIMENTAL

The spot tests for carboxylic acids and esters are the basis for the proposed method (*1*). When an ester is warmed in an alkaline media with hydroxylamine, hydroxamic acid is formed. Feigl (*1*) reports the following reaction:

$$R.CO.OR + NH_2OH = R.CO(NHOH) + ROH$$

Ferric iron forms a bright red or a lavender complex with hydroxamic acids in acid media according to the reaction (*1*):

$$R.CO(NHOH) + Fe^{+++} = R{-}C\begin{matrix}\diagup \\ \diagdown\end{matrix}\begin{matrix}H\\N{-}{-}O\\|\\O\ldots Fe/3\end{matrix}$$

The red colored complex is readily soluble in aqueous ethanol, isopropanol, or methanol, being least stable in the isopropanol. The stability decreases with increased water content, about 5% of water being optimum for the three alcohols.

The ferric hydroxamate complex gains about 0.1% transmittancy per minute for the first 20 minutes but becomes rather stable beyond this, the rate being less than 0.05% per minute. A rise in temperature produces a gain of 0.2% transmittancy per degree at room temperature. The addition of a small amount of sodium carbonate increases the color stability beyond the figures shown above.

Hydrochloric acid and ferric chloride are used in a spot test by Feigl (*1*) to form ferric hydroxamate. The complex follows Beer's law when perchloric acid and ferric perchlorate are substituted for hydrochloric acid and ferric chloride.

Fatty acids do not form hydroxamic acid directly but are first quantitatively methylated in an anhydrous ether solution according to one of the following procedures and the esters determined in the usual way.

With thionyl chloride:

$$R.CO.OH + SOCl_2 = R.COCl + SO_2 + HCl$$

$$R.CO.Cl + CH_3OH = R.CO.OCH_3 + HCl$$

Or with diazomethane:

$$R.CO.OH + CH_2N_2 = R.CO.OCH_3 + N_2$$

Table I. Determination of Oil

Sample No.	Grams of Oil per Base Box	
	Gravimetric	Colorimetric
1	0.67	0.67
2	0.21	0.21
3	0.14	0.15
4	0.18	0.20
5	0.12	0.13
6	2.34	2.30
7	1.67	1.70
8	1.77	1.78
9	2.63	2.50
10	2.10	2.10

SOLUTIONS REQUIRED

Hydroxylamine hydrochloride 2.5% in 95.0% ethanol, made fresh daily.

Sodium hydroxide, 2.5% in 95.0% ethanol, made fresh daily. Saturated with sodium carbonate.

Solution A. Dissolve 0.4 gram of iron or equivalent amount of iron as ferric chloride in 5 ml. of concentrated hydrochloric acid. Add 5 ml. of 70% perchloric acid, evaporate almost to dryness, and dilute to 100 ml. with water. Pipet 10 ml. of this solution to a 1-liter volumetric flask, add 5 ml. of 70% perchloric acid, and make up to mark with 95% ethanol. Prepare weekly.

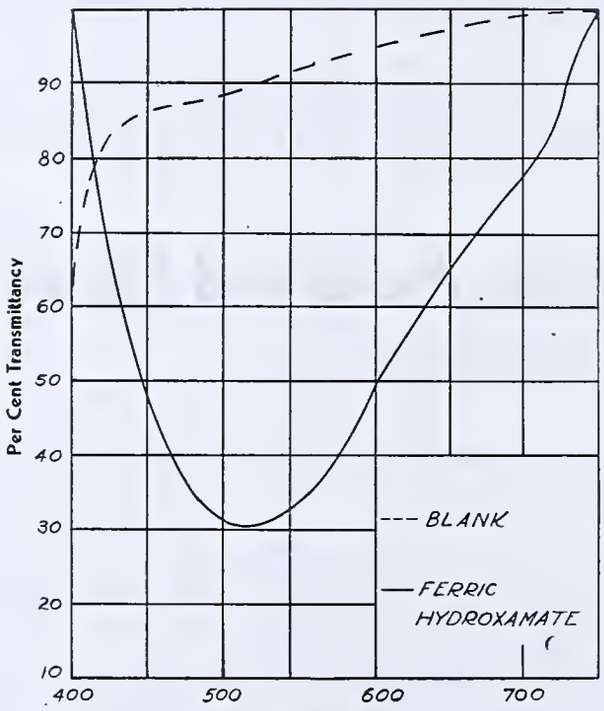

Figure 1. Transmittancy *vs.* Wave Length of Ferric Hydroxamate in 95% Ethanol

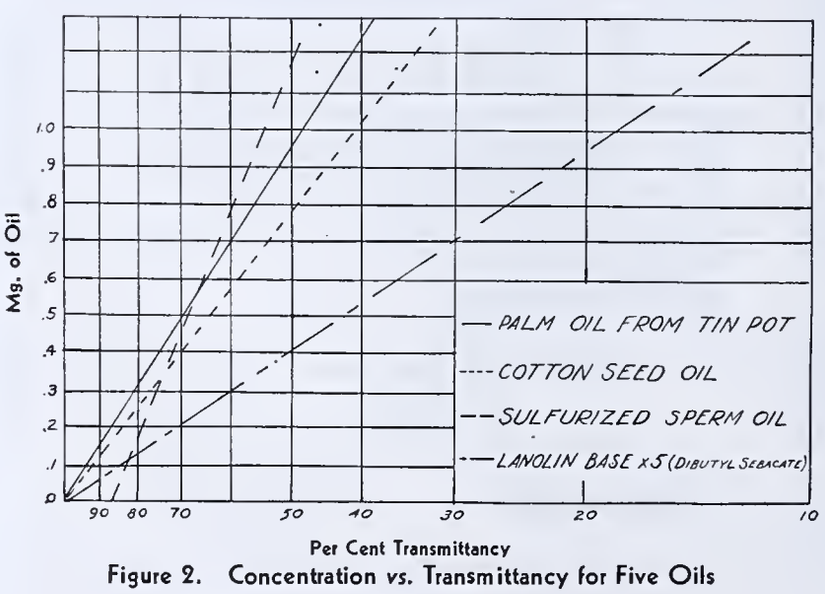

Figure 2. Concentration *vs.* Transmittancy for Five Oils

Solvent for fatty acids and esters, absolute ethyl ether or isopropyl ether free from acids or esters.

Thionyl chloride or a 5% ethereal solution of diazomethane.

Absolute methanol.

METHOD

For Esters. Obtain an absolute ethereal solution in a 250-ml wide-mouthed Erlenmeyer flask containing from 0.05 to 1 mg. of the esters. Add from an accurate transfer pipet 0.3 ml. of 2.5% sodium hydroxide in ethanol and 0.3 ml. of 2.5% hydroxylamine hydrochloride in ethanol. Evaporate to dryness on a water bath at 60° to 70° C. and heat just 5 seconds longer. Add 1 ml. of Solution A, and let stand a few minutes until all salts have dissolved. Prepare a blank in a similar manner. Adjust the temperature to 27° C. and compare transmittancy in a 1.0- to 1.5-cm. cell at 520 mμ with blank. Obtain the amount of ester from a previously prepared ester *vs.* transmittancy curve.

For Fatty Acids. Obtain an ethereal solution containing 0.05 to 1 mg. of fatty acids in 10 ml. of ether, add about 3 ml. of thionyl chloride, and evaporate almost to dryness. Add a few drops of absolute methanol and heat briefly. Evaporate the excess reagents by blowing a gentle current of air into the warm beaker. Add about 20 ml. of anhydrous ether and complete the determination as for esters.

DISCUSSION

Comparison of wave length *vs.* transmittancy against a blank was made on a Coleman Model 11 spectrophotometer having a 35-mμ band. Figure 1 also shows the curve for ferric perchlorate blank. The measurements were made in a 1.5-cm. tube using 10 ml. of solution. Maximum absorption is at 520 mμ.

In adapting the method to Cenco-Sheard-Sanford photelometer, a 515-mμ filter was used with a 1-cm. cell. Sample size ranged from 0.05 to 1 mg. of cottonseed oil.

Because presence of water or alcohol in the ether solution interferes with the color development, anhydrous ether solutions should be used. Technical isopropyl ether was found to contain esters, but was readily purified by saponification with glycerol potassium hydroxide solution followed by simple distillation.

Esterification of fatty acids may be carried out by adding an excess of diazomethane in ether and boiling off the excess. Diazomethane was prepared in sufficiently good purity by the method of Staudinger and Kupfer (*2*). Isopropanol was used instead of absolute ethanol. Since esterification with thionyl chloride was satisfactory, this method was adapted because of its economy despite the superior method of esterification with diazomethane.

The method may be applied to lubricating oils containing fatty esters without preliminary separation. Sulfurized sperm oil was determined in a heavy mineral oil by employing a suitable blank.

Preliminary work has been done on the determination of hydroxyl groups, the esterification being carried out by the use of acetyl chloride in ether solution. After the ether is evaporated, the excess acetyl chloride is destroyed with a few drops of water. After drying, the esters are determined in the usual way.

No attempt has been made to apply the method to the more volatile esters such as ethyl or butyl acetate, but indications are that such determinations may be made by the use of isopropyl ether and a suitable reflux condenser.

RESULTS

The method has been applied in the determination of cottonseed oil on electrolytic tin plate and palm oil on hot-dip tin plate. Lanolin-base oil on black plate has also been determined. Comparative results between gravimetric and colorimetric methods are shown in Table I.

In the gravimetric method, 3 strips 10 cm. wide and 3 cm. long (4 inches wide and 20 inches long, 240 square inches of plate) were formed in a coil and extracted with carbon tetrachloride in a 1-liter Soxhlet extractor for 3

inutes. The extract was filtered and the oil was weighed in tared platinum dish after evaporating off the solvent in a eam bath. For the colorimetric method the oil was extracted om the plate by agitating 9 disks of 26 sq. cm. (4 square inches) ch in a 250-ml. beaker with three 15-ml. portions of ether at om temperature. The reagents were added directly to the her extract. The whole determination was carried out in less an 10 minutes.

Figure 2 shows the transmittancy curves for cottonseed oil, lm oil from tin pots, dibutyl sebacate, sulfurized sperm oil, d a lanolin-base oil used as a rust preventive.

It is apparent that transmittancy values may be plotted in rms of saponification number, acid value, ester value, or acetyl alue.

Larger samples may be used if the corresponding quantities of reagents are used and dilutions made to a greater volume. Thus, a 10-mg. sample of cottonseed oil was determined on the same curve as the 0- to 1-mg. curve by using 1 ml. each of saturated hydroxylamine hydrochloride solution and saturated sodium hydroxide solution and subsequently diluting to 100 ml. with Solution A.

Accuracy of determinations are within ±0.01 mg. of cottonseed oil.

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(2) Staudinger and Kupfer, *Ber.*, 45, 505 (1912).

Determination of Nitrogen, Phosphorus, Potassium, Calcium, and Magnesium in Plant Tissue

Semimicro Wet-Digestion Method for Large Numbers of Samples

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A procedure is described for the determination of nitrogen, phosphorus, potassium, calcium, and magnesium in a single acid-digested sample of plant material. Plant tissue is digested with a salicylic acid–sulfuric acid solution. Nitrogen is determined by nesslerization of the ammonia. Phosphorus is determined by adaptation of the Fiske and Subbarrow (3) method. Potassium is determined by the cobaltinitrite method. The separation of manganese, iron, aluminum, and phosphorus for the determination of calcium and magnesium is made by the method of Peech (7). Calcium is determined by the oxalate method and titrated. Magnesium is precipitated as ammonium phosphate and the phosphate determined as indicated above for phosphorus. An evaluation is made of the analytical errors involved in the determination of the various elements indicated.

INVESTIGATIONS conducted by the Soils Division of the Guayule Research Project on the interrelationships of soil moisture, mineral nutrition, and other factors which affect the growth and rubber formation of guayule have necessitated an extensive study of the nutrient status of guayule plants. Despite the fact that it would be desirable to know the content of all the mineral elements that affect plant growth, it has been necessary to limit the analytical program to the determination of the more common elements—nitrogen, phosphorus, potassium, calcium, and magnesium. No satisfactory method for the determination of all these elements in one sample was found in the literature.

Pepkowitz and Shive (*8*) describe an excellent method for a rapid wet-digestion microdetermination of Kjeldahl nitrogen. They suggest that other elements can be determined in the digest, but give no reference to the elements considered or to the methods which would be suitable for their determination. Parks *et al.* (*6*) describe a method for the microdetermination of 12 elements on one plant sample, but nitrogen is not included. Lindner (*5*) has recently described a method for the determination of nitrogen, phosphorus, potassium, calcium, and magnesium on the same sample of plant tissue, following a digestion in an Erlenmeyer flask with sulfuric acid and hydrogen peroxide. He suggests that care must be taken in the addition of the hydrogen peroxide to prevent the oxidation of ammonia to nitrate.

The authors have found that this is difficult for routine workers to accomplish. The C.P. hydrogen peroxide which is commercially obtainable has the additional disadvantage of containing such large amounts of nitrogen and phosphorus as to necessitate the subtraction of large blanks, or the purification of the hydrogen peroxide. To the authors the use of the sulfuric acid–hydrogen peroxide digestion was not so satisfactory for rapid quantitative work by routine analysis as the method described below.

Numerous methods have been described for the individual determinations of nitrogen, phosphorus, potassium, calcium, and magnesium. Several of the methods for individual determinations have been adapted to a system such that these five elements can be determined in one digest by a rapid and inexpensive procedure. This paper presents a description of this procedure, together with data which make possible the evaluation of the analytical errors involved.

REAGENTS

All reagents are of reagent grade unless otherwise indicated.

DIGESTION. Concentrated sulfuric acid, nitrogen-free.

Ranker's solution, 32 grams of salicylic acid per liter of concentrated sulfuric acid.

Selenium oxychloride, 2.4 grams per liter of concentrated sulfuric acid.

Sodium thiosulfate monohydrate, 50 grams per 100 ml. of distilled water.

Perchloric acid, 10%, diluted from 70% with distilled water.

DETERMINATION OF NITROGEN. Sodium hydroxide, 1 *N*. Gum ghatti, 1%.

Nessler's Reagent. Dissolve 150 grams of potassium hydroxide in 200 ml. of water. Dissolve 50 grams of mercuric chloride in 500 ml. of water. Dissolve 61.75 grams of potassium iodide in 200 ml. of water. Pour the mercuric chloride solution cautiously into the potassium iodide solution until a precipitate begins to form, then add mercuric chloride solution dropwise until a permanent bright red color is produced. Dissolve 0.75 gram of potassium iodide in the red solution, stir well, and transfer to a 1-liter flask. Add the potassium hydroxide solution, shake well, cool, and make to volume. Transfer to a brown bottle and keep in cool, dark place. Centrifuge before using.

DETERMINATION OF PHOSPHORUS. Ammonium molybdate, 2.5% in 5 *N* sulfuric acid.

1,2,4-Aminonaphtholsulfonic Acid. Add 0.125 gram of 1,2,4-aminonaphtholsulfonic acid to 49 ml. of filtered 15% sodium bisulfite, then add 1.25 ml. of 20% sodium sulfite. Mix well.

DETERMINATION OF POTASSIUM. Ethyl alcohol, 95 and 70% by volume.

Sodium Cobaltinitrite. Mix together 46.2 grams of sodium

cobaltinitrite, 18.9 grams of sodium acetate, 120 ml. of distilled water, and 18 ml. of glacial acetic acid. Prepare 48 hours before using. Keep stoppered and in a cold, dark place. Before using, centrifuge to remove any precipitate.

Ceric sulfate, approximately 0.02 *N* in 1 *N* sulfuric acid. Standardize against ferrous ammonium sulfate or sodium oxalate.

Ferrous ammonium sulfate, approximately 0.02 *N* in 0.3 *N* sulfuric acid. Standardize against ceric sulfate.

Indicator, *o*-phenanthroline ferrous complex, 0.025 *M*. Dissolve 0.173 gram of ferrous sulfate and 0.125 gram of *o*-phenanthroline in 25 ml. of water.

Separation of Manganese, Iron, Aluminum, and Phosphorus. Ammonium chloride, 25%; ammonium hydroxide, 0.6 *N*; bromine water, saturated.

Ferric chloride, 1 mg. of iron per ml. Dissolve 1.22 grams of ferric chloride hexahydrate in 250 ml. of water containing 1 ml. of concentrated hydrochloric acid.

Sodium acetate, 10%.

Sodium hydroxide, 0.1 *N*.

Methyl red indicator, 0.02%.

Determination of Calcium. Ammonium acetate, 60%.

Ammonium oxalate, saturated solution.

Ammonium hydroxide, 1%, saturated with calcium oxalate. Centrifuge or filter before using.

Ceric sulfate, ferrous ammonium sulfate and *o*-phenanthroline ferrous complex indicator, as described above under Determination of Potassium.

Sulfuric acid, 1 to 1.

Determination of Magnesium. Ammonium chloride, 25%.

Ammonium dihydrogen phosphate, $NH_4H_2PO_4$, 5%.

Wash Solution. Mix 20 ml. of ammonium hydroxide, 80 ml. of water, 100 ml. of ethyl alcohol, and 100 ml. of ether.

Ammonium molybdate and 1,2,4-aminonaphtholsulfonic acid, as described under Determination of Phosphorus.

2 *N* sulfuric acid.

ANALYTICAL PROCEDURES

Digestion. The digestion of plant material is made by an adaptation of the rapid wet-digestion micro-Kjeldahl method for nitrogen determination described by Pepkowitz and Shive (*8*).

Solution A. Weigh into a 100-ml. Pyrex volumetric flask a suitable sample of the material to be analyzed—for example, 200 mg. of dry guayule tissue. Add 3 ml. of the salicylic acid–sulfuric acid solution, mix it with the tissue, and allow to stand in the cold for about 30 minutes. Add 4 drops of the sodium thiosulfate and 5 ml. of the selenium oxychloride solution. Mix thoroughly. Heat gradually to boiling on a hot plate under a hood and digest until clear. Cool the flasks and wash the necks down with a small stream of water. Cool. If the solution is not colorless, add 2 drops of 10% perchloric acid and shake immediately. Heat again, at low temperature first, and digest until colorless, but do not boil. Cool and make to volume.

Solution B. Shake Solution A thoroughly to resuspend all precipitated material. (Calcium is likely to be precipitated as the sulfate.) Immediately withdraw a 25-ml. aliquot and transfer to a 50-ml. beaker. Evaporate to dryness on a hot plate, and heat in a muffle furnace at a low red heat, to drive off ammonia salts and excess sulfuric acid. Cool and take up with 2 drops of concentrated hydrochloric acid and 10 ml. of water. Transfer quantitatively to a 25-ml. volumetric flask and make to volume.

Determination of Nitrogen. Nitrogen is determined by the nesslerization of ammonia according to the procedure described by Fraps and Sterges (*4*).

After the aliquot for Solution B has been removed and the suspended material in Solution A has settled, quantitatively transfer 2 ml. Solution A to a 100-ml. volumetric flask. Add 50 to 60 ml. of water, then 2 ml. of 1 *N* sodium hydroxide, and shake thoroughly. Add 2 ml. of 1% gum ghatti and shake again. Add 2 ml. of Nessler's reagent, shake thoroughly, and make to volume. Using a 420-mu filter, compare in a colorimeter with a series of standard ammonium hydroxide solutions treated similarly.

Determination of Phosphorus. Phosphorus is determined by an adaptation of the method of Parks, Hood, Hurwitz, and Ellis (*6*) and Fiske and Subbarrow (*3*).

After the aliquot for Solution B has been removed and Solution A has settled until clear, transfer 5 ml. of Solution A to a 50-ml. volumetric flask. Add 5 ml. of ammonium molybdate solution and shake. Add 2 ml. of 1,2,4-aminonaphtholsulfonic acid solution, make to volume, and shake well. Prepare a set of standards simultaneously, since the slope of the standard curve is not constant (*6*). Allow the color to develop 30 minutes. Read in a photoelectric colorimeter, using a filter of 660-mu wave length for color comparison.

Determination of Potassium. Potassium is determined by a modification of the method of Brown, Robinson, and Brownin (*2*).

To 1.5 ml. of 95% ethyl alcohol in a 15-ml. centrifuge tube ad a 5-ml. aliquot of Solution B and mix throughly. Place the tub in a water bath at 20° C. and allow to come to equilibrium. Ad slowly, with continuous shaking, 2 ml. of sodium cobaltinitrit solution, and allow to stand for exactly 1 hour at a temperatur of 20° ± 1° C. Centrifuge for 10 minutes at 2000 r.p.m decant the supernatant liquid, invert the tube at an angle about 45°, and let drain for several minutes. Wash the pre cipitate with 5 ml. of 70% alcohol, centrifuge for 5 minutes, an drain as before. Dry the precipitate at 80° C. until all the alcoh is removed. Add 5 ml. of 0.02 *N* ceric sulfate (if more tha 0.5 mg. of potassium is present, more than 5 ml. of ceric sulfat is required) and 1 ml. of 1 to 1 sulfuric acid. Heat in a water bat at 90° to 95° C. until all the precipitate is oxidized, cool, an titrate the excess ceric sulfate with 0.02 *N* ferrous ammoniur sulfate, using 1 drop of the *o*-phenanthroline ferrous comple indicator. The end point is very sharp, the color of the solutio changing from pale blue to red.

A set of standard potassium solutions, containing approxi mately the same amount of potassium as is present in the ali quots of Solution B, should be carried through the procedure a the same time. Assuming complete recovery of potassium in th standard solution, calculate a factor for the equation:

$$\text{Mg. of potassium} = \text{ml. of } Ce(SO_4)_2 \text{ used in oxidation of precipitate} \times \text{normality of } Ce(SO_4)_2 \times \text{factor}$$

Calculate the potassium content of the digested plant materia (Solution B), using the calculated factor in the same equation.

Separation of Manganese, Iron, Aluminum, and Phosphorus. Manganese, iron, aluminum, and phosphorus are re moved according to the method of Peech (*7*).

Solution C. Transfer a 5-ml. aliquot of Solution B to a 15-ml centrifuge tube, and add 0.2 ml. of ferric chloride solution, 3 ml of water, and 2 ml. of 10% sodium acetate solution. Mix, add ml. of 0.1 *N* sodium hydroxide, and mix again. Place in a wate bath at 95° C., add 1 ml. of bromine water, and maintain thi temperature for at least an hour. Add 2 ml. of 25% ammoniun chloride solution and digest about 15 minutes longer. Add drop of methyl red and if the color of the indicator persists indicating complete expulsion of bromine, remove the tube fron the water bath, cool, add 0.6 *N* ammonium hydroxide from buret until the color of the solution changes from slightly red t deep yellow, and then add 2 drops in excess. Make to volume o 13 ml., add 5 drops of water in excess to allow for evaporation mix the contents with a stirring rod, and digest in a water bath at 80° C. for 5 minutes to flocculate the precipitate. Whil hot, centrifuge for 10 minutes.

Determination of Calcium. Calcium is determined by modification of the method of Peech (*7*).

Solution D. Without disturbing the precipitated manganese iron, aluminum, and phosphate, transfer 10 ml. of Solution C t a 15-ml. centrifuge tube, and add 1 ml. of 60% ammonium ace tate. Place the tube in a water bath at 70° C. Mix the con tents by spinning the tube, add 2 ml. of saturated ammonium oxalate solution, mix thoroughly, and digest for 30 minutes Cool, make to volume of 13 ml., and centrifuge for 10 minute at 2000 r.p.m. Decant the supernatant liquid into a dry tes tube and save for the determination of magnesium.

Allow the centrifuge tube, inverted at about a 45° angle, t drain for several minutes. Wipe the lip of the tube with filte paper. Wash the precipitate with 5 ml. of a 1% ammonium hydroxide solution saturated with calcium oxalate and centri fuge again. Decant and discard the supernatant liquid. Allow the tube to drain again for several minutes. Add 3 to 5 ml. o 0.02 *N* ceric sulfate (depending upon the amount of calcium pres ent) and 1 ml. of 1 to 1 sulfuric acid. Mix, heat to 70° C. in water bath, and titrate with 0.02 *N* ferrous ammonium sulfate using 1 drop of *o*-phenanthroline ferrous complex indicator.

Determination of Magnesium. Magnesium is determine by an adaptation of the method of Reitemeier (*9*).

Transfer 5 ml. of Solution D to a 15-ml. centrifuge tube. Ad 1 ml. of 25% ammonium chloride, 1 ml. of 5% ammonium dihy drogen phosphate, and 1 drop of phenolphthalein. Mix, hea to 90° C. in a water bath, and add concentrated ammonium hy droxide until pink. Cool, add 2 ml. of concentrated ammonium hydroxide, and stir with a glass rod. Withdraw the rod and rins it with a very small stream of water. Stopper the tube and le it stand overnight (preferably in a cool place). Centrifuge, de cant, invert the tube at about a 45° angle, and let drain severa minutes. Wash the precipitate and sides of the tube with 5 ml of the ammonia-alcohol-ether wash solution. Decant and drain

Table I. Determination of Nitrogen, Phosphorus, Potassium, Calcium, and Magnesium on Standard Guayule Plant Tissue[a] with Added Increments of These Elements

Sample	Nitrogen				Phosphorus				Potassium				Calcium					Magnesium			
	Added	Source	Total present	Found	Recovery	Added	Total present	Found	Recovery	Added	Total present	Found	Recovery	Found A	Found B	Found C	Mean	Recovery	Added	Total present	Found	Recovery
	Mg.		*Mg.*	*Mg.*	%	*Mg.*	*Mg.*	*Mg.*	%	*Mg.*	*Mg.*	*Mg.*	%	*Mg.*	*Mg.*	*Mg.*	*Mg.*	%	*Mg.*	*Mg.*	*Mg.*	%
Plant tissue	0	....	6.50	...	...	0	...	0.69	...	0	...	8.00	...	5.22	5.14	5.43	5.26	...	0	...	1.11	...
	0	....	6.35	...	...	0	...	0.69	...	0	...	8.34	...	5.39	5.47	5.34	5.40	...	0	...	1.15	...
	0	....	6.40	...	...	0	...	0.69	...	0	...	8.12	...	5.39	5.43	5.30	5.37	...	0	...	1.25	...
	..	....	...	...	...	..	...	..	...	0	...	...	...	5.34	5.43	5.59	5.45	...	0	...	1.25	...
	..	....	...	...	...	..	...	..	...	0	...	...	...	5.47	5.63	5.39	5.50	...	0	...	1.28	...
	..	....	...	...	...	..	...	..	...	0	...	...	...	5.14	5.55	5.34	5.34	...	..	..	..	...
Av.	0	....	6.42	...	100	0	..	0.69	100	0	...	8.15	100	5.33	5.44	5.40	5.39	100	0	..	1.21	100
Plant tissue	3.00	KNO_3	9.40	9.90	105	1.01	1.70	1.68	99	2.80	10.95	11.14	102	7.30	7.75	7.51	7.52	102	1.00	2.21	2.40	109
	3.00	KNO_3	9.40	9.80	104	1.01	1.70	1.69	99	2.80	10.95	11.12	102	7.84	7.59	7.34	7.42	100	1.00	2.21	2.27	103
	3.00	KNO_3	9.40	10.10	107	1.01	1.70	1.67	98	2.80	10.95	11.08	101	7.22	7.34	7.34	7.30	99	1.00	2.21	2.28	103
	..	....	...	...	...	..	...	..	...	...	...	...	...	...	...	...	...	...	1.00	2.21	2.37	107
	..	....	...	...	...	..	...	..	...	...	...	...	...	...	...	...	...	...	1.00	2.21	2.23	101
Av.	3.00	KNO_3	9.40	9.90	105	1.01	1.70	1.68	99	2.80	10.95	11.11	101	2.00	7.39	7.56	7.40	7.41	100	1.00	2.21	2.31	105
Plant tissue	6.00	KNO_3	12.42	12.70	102	0.68	1.37	1.35	98	8.30	16.45	16.30	99	4.00	9.39	9.10	9.18	9.38	9.22	98	3.00	4.21	4.33	103
	6.00	KNO_3	12.42	13.00	105	0.68	1.37	1.35	98	8.30	16.45	16.40	100	4.00	9.39	9.10	9.38	9.38	9.29	99	3.00	4.21	4.20	100
	6.00	KNO_3	12.42	12.60	101	0.68	1.37	1.37	100	8.30	16.45	16.25	99	4.00	9.39	9.18	9.02	9.06	9.09	97	3.00	4.21	4.17	99
	..	....	...	...	...	..	...	..	...	...	...	...	...	...	...	...	...	...	...	...	3.00	4.21	4.53	108
	..	....	...	...	...	..	...	..	...	...	...	...	...	...	...	...	...	...	...	...	3.00	4.21	4.40	105
	..	....	...	...	...	..	...	..	...	...	...	...	...	...	...	...	...	...	...	...	3.00	4.21	4.33	103
Av.	6.00	KNO_3	12.42	12.80	103	0.68	1.37	1.36	99	8.30	16.45	16.32	99	4.00	9.39	9.13	9.19	9.27	9.20	98	3.00	4.21	4.33	103
Plant tissue	2.50	NH_4Cl	8.92	8.80	98	0.34	1.03	1.02	99	16.70	24.85	24.60	99	6.40	11.79	11.51	11.51	11.71	11.58	98	..	..	..	...
	2.50	NH_4Cl	8.92	8.65	97	0.34	1.03	1.03	100	16.70	24.85	24.00	97	6.40	11.79	11.14	11.63	11.73	11.50	98	..	..	..	...
	2.50	NH_4Cl	8.92	8.80	98	0.34	1.03	1.02	99	16.70	24.85	22.80	92	6.40	11.79	11.42	11.26	10.89	11.19	95	..	..	..	...
Av.	2.50	NH_4Cl	8.92	8.72	98	0.34	1.03	1.02	99	16.70	24.85	23.80	96	6.40	11.79	11.36	11.47	11.44	11.42	97	..	..	..	...
Plant tissue	5.00	NH_4Cl	11.42	10.50	92	0.17	0.86	0.84	98	...	...	...	...	...	...	...	...	...	...	...	..	..	..	...
	5.00	NH_4Cl	11.42	11.75	103	0.17	0.86	0.85	99	...	...	...	...	...	...	...	...	...	...	...	..	..	..	...
	5.00	NH_4Cl	11.42	11.60	102	..	..	..	...	...	...	...	...	...	...	...	...	...	...	...	..	..	..	...
Av.	5.00	NH_4Cl	11.42	11.28	99	0.17	0.86	0.84	98	...	...	...	...	...	...	...	...	...	...	...	..	..	..	...

[a] 200 mg. of plant tissue used in each determination.

Repeat this washing and draining once. Dissolve the precipitate with 5 ml. of 2 *N* sulfuric acid, transfer to a 25-ml. volumetric flask, and develop the phosphate color as directed above under Determination of Phosphorus. Prepare a photometric calibration curve by simultaneously carrying a series of standard magnesium sulfate solutions through the same operations each time a group of samples is analyzed. From this curve calculate the amount of magnesium present in the unknowns.

RESULTS AND DISCUSSION

The length of time required for the digestion of any given sample of plant material will depend upon the temperature at which the digestion takes place. If kept boiling vigorously, the digestion will usually be completed within 1 to 2 hours. Pepkowitz and Shive (*8*) reported that 10 to 25 minutes were required for the complete digestion of samples of 10 to 15 mg. of plant material. The time required for complete digestion of 200-mg. samples of guayule tissue for a routine analysis in this laboratory is usually 3 to 6 hours. If boiling temperatures are not maintained, 8 to 16 hours may be required. The sulfuric acid digestion can be continued until the digest is colorless, thus eliminating the use of perchloric acid. This usually requires 3 to 6 hours longer and is not a recommended practice. As is pointed out by Pepkowitz and Shive, the digest should not be boiled after the perchloric acid has been added. Boiling at this stage may result in a loss of nitrogen through the oxidation of ammonia to nitrate.

At first thought it may appear that a method which requires 3 to 6 hours' digestion is not a rapid method for the analysis of plant tissue. Little attention is required during the digestion process, however, and in the analysis of large numbers of samples the time required for digestion is of little consequence if the work is properly planned. Only one weighing and digestion is required for the determination of the five elements most commonly determined. (In this laboratory rapid weighing of samples is facilitated by the use of a Roller-Smith microtorsion balance of 0- to 1500-mg. capacity.) The analyst can proceed with determinations on the digested material while a second set of samples is digesting. The gas hot plate used in this laboratory is 45 × 90 cm. (18 × 36 inches) in dimension and will accommodate 105 100-ml. volumetric flasks. Chemical determinations on the digests that can be made with a hot plate of this size will more than keep two good routine analysts busy.

In this laboratory a digest and reagent blank, and duplicates of a standard guayule sample, are carried through the entire analytical procedure with each lot of samples. The digestion and reagent blank allow for correction for impurities in the reagents, which may vary with changes in sources. The standard guayule sample which is analyzed with each run serves as a check on accuracy. For this purpose it is preferred to a synthetic standard.

It is necessary to shake Solution A thoroughly before taking the 25-ml. aliquot for the determination of potassium, calcium, and magnesium. It is equally essential that that aliquot be taken before any other aliquots are removed from Solution A, unless the solution be thoroughly shaken before such aliquots are taken. Because of the low solubility product of calcium sulfate, and the high concentration of sulfuric acid in the digest, calcium is likely to be present as precipitated calcium sulfate. If this precipitate is not uniformly suspended throughout the solution by shaking, the aliquot for potassium, calcium, and magnesium will not be representative. Likewise, if the aliquot for nitrogen or phosphorus be taken from the flask without thoroughly mixing the

contents there will be a concentration of calcium sulfate in the remaining solution; and any subsequent sample, if then representative of what remains in the flask, will contain a greater concentration of calcium than the original digest. For these reasons it is advisable to take the aliquot for potassium, calcium, and magnesium first and then allow the suspended material in Solution A to settle before the aliquots for nitrogen and phosphorus are taken.

The reliability of the procedures used is indicated by the data of Table I. Varying amounts of nitrogen, phosphorus, potassium, calcium, and magnesium were added to standard guayule tissue, before digestion, and the recovery of each was determined. The data given are typical of several sets of determinations.

The recovery of nitrogen added to guayule tissue varied from 92 to 107%. These were extreme values, however, and in most cases the recovery was between 97 and 103%.

The recovery of added increments of phosphorus was very satisfactory. In the described procedure silica is not removed. If silicates are present to any considerable extent, it may be necessary to transfer an aliquot of Solution A to a platinum dish and treat it with hydrofluoric acid to remove the silica before proceeding with the determination of phosphorus.

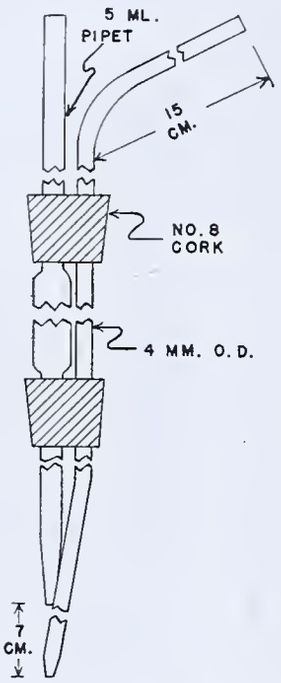

Figure 1. Pipet Assembly for Washing Compacted Precipitates in Centrifuge Tubes

The recovery of potassium was very good except where large amounts were added. In nearly all cases a recovery of 97 to 103% was obtained. The determination of potassium by the cobaltinitrite method must take place under standardized conditions. It is necessary that the precipitation be carried out at nearly constant temperature, preferably 20° ± 1° C. The potassium content of the aliquot for precipitation should be between 0.2 and 1.0 mg. During centrifugation the precipitate compacts tightly in the bottom of the centrifuge tube. It is rather difficult to wash such a compacted precipitate well. The authors have found that a pipet assembly constructed as shown in Figure 1 aids considerably in the washing of precipitates compacted in the bottom of conical centrifuge tubes.

The pipet is filled with the wash solution and the small glass tube is placed in contact with the compacted precipitate. As the wash liquid drains from the pipet, air is blown through the glass tube, thus thoroughly mixing the precipitate with the wash solution. Before all the wash solution has drained from the pipet the glass tube is raised clear of the liquid in the centrifuge tube. The remaining liquid draining from the pipet then washes the small tube free of any precipitate or solution that may have adhered to it. The side of the centrifuge tube may also be washed by focusing the wash liquid around the top inside edge. This type of pipet assembly is used with all precipitates being washed by centrifugation.

The composition of the precipitate obtained in the volumetric determination of potassium by the sodium cobaltinitrite method varies, depending upon the conditions of precipitation, particularly temperature, concentration of alcohol, and concentration of sodium (*10*). Brown, Robinson, and Browning (*2*) used an empirical factor of 6.52 in the calculation of the potassium in the precipitate. In the authors' studies seldom did calculations made with the use of this factor indicate 100% recovery of the potassium present in standard solutions of potassium nitrate. In this laboratory the procedure was adopted of carrying standard potassium solutions through the precipitation and titration procedure simultaneously with each set of unknowns. Assuming 100% recovery of potassium from the potassium standards, an empirical factor was calculated for the conditions under which the precipitation was carried out. This factor was then used in the calculation of the potassium content of the unknowns. Although it has been demonstrated (*2*) that calcium, magnesium, iron, sulfate, chloride, phosphate, and certain other ions do not interfere in the volumetric determination of potassium with cobaltinitrite, it is advisable to have the standard solution contain these ions in about the same ratios as they are present in the plant material.

In Table I are presented the data obtained in the analysis of guayule with added increments of calcium as calcium chloride. Determinations were made on three aliquots from each of six digestions of guayule with no added calcium, and from each of three or more digestions with increments of calcium. It is evident that the variation between aliquots from the same digestion is of about the same magnitude as that between digestions, and that this variation is not great. For the 18 determinations made on guayule tissue alone the coefficient of variation is 2.5%.

The maximum variation in the percentage recovery of the various elements added to the standard guayule tissue occurred in the determination of magnesium. It is thought that most of the error involved is in the actual determination of magnesium and is not in any way due to the digestion procedure. The authors have not found a completely satisfactory method for the colorimetric determination of small amounts of magnesium.

The accuracy of the determination of phosphorus and potassium by the digestion procedure described in this paper was checked by the ashing methods of the A.O.A.C. (*1*). Five samples were analyzed in duplicate by each method (Table II). The phosphorus data obtained by the two methods are in good agreement, the maximum variation amounting to about 6%. The potassium data agree satisfactorily, but are somewhat more variable than those for phosphorus, the maximum difference being about 8%.

Table II. Comparison of Ashing Method of A.O.A.C. and Digestion Method for Determination of Phosphorus and Potassium in Guayule Tissue

Sample No.		Per Cent P, Dry Basis		Per Cent K, Dry Basis	
		Ash (A.O.A.C.)	Digestion	Ash (A.O.A.C.)	Digestion
1		0.21	0.21	6.7	7.0
		0.22	0.22	6.7	6.9
	Av.	0.22	0.22	6.7	7.0
2		0.28	0.29	7.0	7.1
		0.30	0.30	7.3	7.3
	Av.	0.29	0.30	7.2	7.2
3		0.27	0.27	7.0	6.5
		0.26	0.25	6.8	6.2
	Av.	0.27	0.26	6.9	6.4
4		0.20	0.21	7.1	6.6
		0.20	0.22	7.1	7.0
	Av.	0.20	0.22	7.1	6.8
5		0.19	0.20	5.8	6.4
		0.20	0.21	6.3	6.4
	Av.	0.20	0.21	6.1	6.4

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Use of an Ion-Exchange Resin in Determination of Traces of Copper

With Special Reference to Powdered and Fluid Milk

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A simple and rapid determination of copper in fluid milk, powdered milk, and ice cream mix is described. Ignition or acid digestion of the sample is eliminated, the copper being made available by reducing the pH with perchloric acid. Copper is then concentrated on an ion exchanger in the hydrogen cycle, stripped from the ion exchanger with dilute hydrochloric acid, and determined polarographically using an additive standard technique.

THE chief difficulty encountered in determining the copper content of milk is the extremely small concentration of the metal present. Raw milk has been reported from several sources to contain from 0.1 to 0.5 p.p.m.; the Quartermaster Corps limits the copper content of premium grade powdered whole milk to 1.5 p.p.m. and nonfat milk solids to 2.0 p.p.m. The usual copper content of premium grade powdered whole milk is now found to be less than 1.0 p.p.m.

After exhaustive investigation of applicable methods, a spectrophotometric method employing dithizone (*2*) has been selected as the official army specification method of analysis, as being the most sensitive and specific. However, the specification method lacks precision. Three laboratories determined copper on six samples of powdered whole milk ranging from 0.6 to 20.0 p.p.m. Each laboratory made ten determinations on each sample and the coefficient of variation was determined for each sample for each laboratory. Figure 1 demonstrates that the coefficient of variation values becomes excessively large at very low concentrations, particularly at the critical range below 2.0 p.p.m. Thus is demonstrated the necessity of making replicate determinations on all borderline samples. Seven points were not plotted. Three represented data obtained from less than ten determinations each, and four represented high coefficient of variation values on 0.5 and 1.0 p.p.m. samples that were above 22 and off the scale. However, all seven points not plotted appeared to fit the curves well.

By and large, the limiting factor in the determination of the copper content in dairy products or, for that matter, in food products in general is the size of the sample that can be adequately handled. In practically all methods of analyses it is necessary to destroy the organic matter as the primary step. Whether the destruction is accomplished by acid digestion or ignition, the procedure when applied to low copper content foods of high protein and fat content becomes both laborious and time-consuming. A further handicap is that only a relatively small sample is practical, and the resulting concentration of copper in the prepared sample is also exceedingly small. The ideal procedure would be one in which preliminary ashing is eliminated and a sufficiently large sample can be taken to ensure an adequately measurable concentration of copper.

Recently it has been shown (*1*) that copper proteins lose their copper in acid solution below pH 3.0. Both cupric and cuprous copper are lost, the latter being oxidized in the acid aqueous solution (*6*). It has also been demonstrated that copper in powdered whole milk is concentrated in the milk protein (*10*). Presumably a similar condition exists in raw milk. It is therefore sufficient to transfer the copper in milk to the electrovalent form of cupric ions by reducing the pH to less than 3.0. This accomplishes a secondary advantage, in that under optimum conditions the protein is precipitated and fat will be carried into the curd. Thus by simple filtration it is possible to make the copper available and to eliminate interfering protein and fat. The filtrate so obtained, when corrected for added acid, solids and fat loss, contains copper in the same concentration as the original solution whether it was raw milk or reconstituted powdered milk. Synthetic ion-exchange resins offer a satisfactory method of concentrating the copper from the milk filtrate with the minimum effect of residual organic constituents remaining in the milk filtrate.

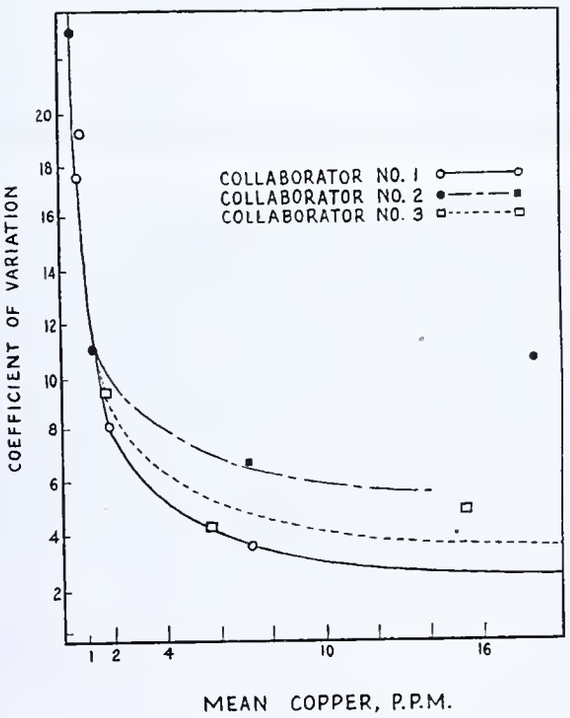

Figure 1. Reproducibility of Data Obtained by Bendix-Grabenstetter Procedure, Employing 5-Gram Samples

While there has been a considerable amount of work published on the general behavior of ion-exchange resins on cations in solution (*4, 8*), very little has been published on concentrations for analytical procedures. Zeo-Carb has been used (*3*), but it was not considered satisfactory for trace work because it cannot be purified sufficiently. In so far as the authors are aware there have been no publications describing the use of ion-exchange resins as a means of concentrating metals in microgram concentrations. The general equation applicable to the separation of cations from anions by any ion exchanger in the hydrogen cycle (*3*) may be represented as:

$$2H^+ A^- + Cu^{++} + SO_4^{--} \rightleftharpoons Cu^{++} A^-_2 + 2H^+ + SO_4^{--}$$

EXPERIMENTAL

The usual protein precipitants such as metaphosphoric, trichloroacetic, and tungstic acids were unsatisfactory, owing to

the difficulty involved in removing traces of copper from these reagents. Glacial acetic acid, although a satisfactory precipitant, could not be used because of buffering capacity of the system. An unreasonably large amount was required to reduce the pH substantially below pH 3.5. An ideal precipitant was found in perchloric acid (*7*); an almost negligible amount was required to reduce the pH to 1.0 with a resulting precipitate that was easily filtered. The data presented in Table I were obtained from samples by varying the contact time of the perchloric acid with the milk from 30 minutes to 16 hours and demonstrate the rapidity with which the copper is removed from protein complexes at low pH. Complete liberation is obtained in 30 minutes or less.

Table I. Spectrophotometric Data Obtained by Acid Digestion of Samples and Prepared Filtrates

Sample	Copper Content of 5-Gram Sample	Copper content of 75-Ml. Prepared Filtrate
	P.p.m.	*P.p.m.*
Raw fluid milk	0.15	0.10
	0.15	0.15
Powdered whole milk	0.85	0.70
	0.80	0.80
	0.95	0.85
	0.95	0.90
	0.60	0.70
Powdered whole milk, special	15.0	15.5
Duplicate determination	15.2	15.0
	...	15.2
Ice cream mix, powdered	0.20	0.35
	0.65	0.45

No attempt has been made to compare all the possible ion exchangers. Amberlite IR-100 has been used entirely for this study and has been found entirely satisfactory. The resin was supplied as the hydrogen derivative, and was used in the hydrogen cycle. However, as the resin supplied in its original form contained a small amount of copper it had to be purified. This was easily accomplished by washing with 10% hydrochloric acid followed by a water rinse. Back-washing was not necessary, down flow of both regenerate and rinse being employed. In using the proposed method zero copper blanks were obtained. Dry-ashing of the prepared resin indicated a residual copper content of much less than 1 microgram per gram, but as this copper was not removed by the elutriant acid and rinse it can be ignored. A column of resin 150 mm. long and 12 mm. in diameter absorbed up to 1 mg. of copper regardless of the amount of filtrate in which it was dissolved. Thus, a column of this size was more than adequate for absorbing all the copper in 100 ml. of milk filtrate. Calcium absorption did not interfere, even though the concentration was high—up to 250 mg. per 100 ml. of milk. Passage of filtrate through the column was rapid and a rinsing with 100 ml. of water was found to be sufficient. The copper absorbed by the resin was readily dissolved in dilute hydrochloric acid.

Polarographic analysis of the elutriant was employed as offering the most rapid and simple method. This does not preclude spectrophotometric methods of analyses, however.

METHOD OF APPLICATION

Apparatus. Suitable polarograph with cells of approximately 10-ml. capacity.

Pyrex Ware. All Pyrex ware should be thoroughly cleaned by washing with concentrated nitric acid, and rinsed thoroughly with tap water, with distilled water, and finally several times with redistilled water. Columns can be prepared from Pyrex condenser tubes, chromatographic separation tubes, filter tubes, etc. It is essential only that a column should be approximately 150 mm. × 10 mm. in diameter. The lower end of the column should be plugged loosely with just sufficient glass wool to retain the resin. The elutriant should be withdrawn through a small length of amber gum rubber tubing equipped with a screw clamp to shut off and to adjust flow of elutriant.

Reagents. Amberlite IR-100, analytical grade, low in copper, obtained from Resinous Products and Chemical Company, Philadelphia, Pa.

Redistilled Water. Distilled water redistilled from Pyrex.

Perchloric Acid, 72%, double vacuum-distilled, obtained from G. Frederick Smith Chemical Company, Columbus, Ohio.

Filter Paper. Wash with dilute hydrochloric acid and rinse with redistilled water to remove any traces of copper.

Cresol Purple Indicator. Dissolve 0.1 gram of cresol purple in ethyl alcohol.

Ammonia, Dilute. Dilute 10 ml. of concentrated ammonium hydroxide with 100 ml. of redistilled water.

Hydrochloric Acid, 6%. Dilute 1 volume of concentrated hydrochloric acid with 5 volumes of redistilled water.

Electrolyte. 3 *N* ammonia and ammonium chloride with gelatin as a maxima suppressor. Prepare as follows:

NH_4OH (28%)	200 ml.
NH_4Cl	40 grams
Gelatin	10 ml. of a 1% solution

Make up to 1000 ml. with redistilled water.

Standard Copper Solutions. Stock Solution. Dissolve 1.0000 gram of electrolytic sheet copper in 20 ml. of concentrated nitric acid and make up to 1000 ml. with redistilled water. One milligram of this solution is equivalent to 1 mg. of copper.

Working Standard. Dilute a volume of the stock solution with redistilled water so that 1 ml. of the working standard is equivalent to 20 micrograms of copper. Prepare a fresh working standard daily.

Wash the untreated Amberlite IR-100 in a 1000-ml. Erlenmeyer flask with a fairly strong stream of tap water. Allow flask to overflow, adjusting the water pressure so that any undue amount of fine material will be carried off with the overflow. Transfer the washed resin to a suitable column of such capacity that a considerable quantity of resin may be freed of copper at one time. The column must be filled with water prior to the addition of the resin. Fill column so that it is about three-quarters full of resin. Drain water in the column so that only a few millimeters height remains above the resin bed.

Slowly pass hot 10% hydrochloric acid through the resin, allowing the fluid level almost to reach the surface of the resin before adding the next portion. Continue the acid wash until 75 ml. of the effluent acid wash show a negative copper content when tested with a copper-specific reagent such as carbamate or dithizone. Wash free of hydrochloric acid with redistilled water as indicated by a negative chloride test with silver nitrate.

Store the prepared resin under redistilled water.

A suitable column is easily prepared if it is first filled with redistilled water and the resin added. Never allow the resin to become dry. In all operations make certain that the fluid level never passes below the upper level of the resin. Properly cleansed resin will give zero copper contents when washed with a water–perchloric acid solution at pH 5.0.

Sample Preparation. Whole Milk. Mix well to ensure uniform distribution of fat.

Powdered Milk and Ice Cream Mix. Reconstitute 25 grams in redistilled water and make up to 250-ml. volume with redistilled water. Reconstitution is best accomplished by adding the milk slowly to water with constant agitation.

Procedure. Transfer the prepared sample to a 400-ml. beaker, add 4.00 ml. of double vacuum-distilled 72% perchloric acid, and mix thoroughly by stirring. Allow to stand at least 0.5 hour. Stir thoroughly but gently and filter. Allow the precipitated curd to drain; the curd should retain no more than 25% of the filtrate. If the filter paper is washed free of copper just prior to use, discard the first few milliliters of filtrate which will be diluted with water from the paper. Transfer 100 ml. of filtrate to a 150- to 250-ml. beaker, and add 3 drops of cresol purple indicator. Add dilute ammonia reagent slowly with thorough mixing until the addition of one drop yields a change from yellow to gray or gray-purple (approximately pH 5.0). At this point there will be formed a slight but permanent precipitate, which may be utilized as a further indication of the end point.

Pass the neutralized filtrate through a prepared ion-exchanger column, adjusting the flow so that the dropping rate is 2 to 3 drops per second. Never allow the ion exchanger to become dry. Always stop flow so that a small amount of fluid remains above the surface. Rinse residual filtrate from the beaker into the column with redistilled water and wash the column with about 200 ml. of redistilled water. Stop flow on final wash, so that about 5 mm. of water remain above the resin.

Wash the absorbed copper from the column with a total volume of 40 ml. of 6% hydrochloric acid, using four approximately equal portions. Never allow fluid level to pass below top level of resin bed. On the final wash allow the column to drain as completely as possible. Evaporate the combined washings to dryness on a water bath and cool. Add 5 ml. of the electrolyte, stir, and allow to stand 10 minutes. Prepare two polarographic cells. To cell *A* add 1 ml. of redistilled water and 2 ml. of the prepared sample. To cell *B* add 1 ml. of the working copper standard (1 ml. = 20 micrograms of copper) and 2 ml. of the prepared sample. Eliminate oxygen by bubbling hydrogen or nitrogen

through the cells for 5 minutes and record polarograms. Run a reagent blank daily on redistilled water and perchloric acid and correct for any copper found in the reagents. Run the blank in the same manner as the sample.

CALCULATION. Let a represent the step height due to the concentration of copper in cell A (unknown + water) and b the step height due to the concentration copper in cell B (unknown + standard). The difference $(b - a)$ is then due to the added standard which represents a concentration of $^1/_3$ of 20 or 6.67 micrograms of copper per ml. Then $\left(\frac{a}{b-a}\right) \times \left(\frac{20}{3}\right)$ is the concentration of the copper in the unknown in micrograms per ml. $\left[\left(\frac{a}{b-a}\right) \times \frac{20}{3} \times \frac{3}{2} \times 5\right]$ or $\left[\left(\frac{a}{b-a}\right) \times 50\right]$ represents the total micrograms copper in the aliquot of milk filtrate taken for analysis. (It is assumed, of course, that the analyst will determine a and b at the same sensitivity.)

Determine the micrograms of copper in the milk filtrate as directed above. Deduct for a reagent blank and report copper on a p.p.m. basis of the original sample.

Example: Make up 25 grams powdered milk to 250 ml. and take 100 ml. of filtrate for analysis; 100 ml. of filtrate would then represent 10 grams of sample. If step heights a and b are found to be 55 and 75, respectively, the total micrograms of copper in the 100 ml. of filtrate would be $\left[\left(\frac{55}{75-55}\right) \times 50\right]$ or 137.57 micrograms of Cu.

If the reagent blank was found to contain 5 micrograms of copper, the copper expressed as p.p.m. would be $\left(\frac{137.57-5.0}{10}\right)$ or 13.3 p.p.m. (micrograms per gram).

Express copper in powdered products as p.p.m. (mg. per kg.) and in fluid milk as p.p.m. (mg. per liter).

Table II. Precision of Proposed Method

(Single sample of high copper content powdered whole milk)

Aliquot of Prepared Filtrate	Added Copper	Sensitivity	Step Height	Total Copper Found in Aliquot	Copper Originally Present in Filtrate
Ml.	γ		$^1/_{10}$ *in.*	γ	γ/*ml.*
100	0.0	1/5	73	114.50	1.14
50	40.0	1/5	62	97.25	1.14
100	0.0	1/10	35	112.00	1.12
50	40.0	1/10	30	96.00	1.12

Recovery of copper from aqueous perchloric acid solution, pH 5.0

Copper passed through column	80.0 γ
Copper recovered from column	79.2 γ
Copper passed through column	10.0 γ
Copper recovered from column	9.9 γ

DISCUSSION

When the procedure was first tried, an external standard was employed, but the amount of copper recovered was erratic. This condition was corrected by using an internal standard or "standard addition" technique as described by Hohn (*5*). The failure of the external standard method was traced to the fact that small but varying quantities of precipitated casein redissolved at the very low pH employed. On neutralization of the filtrate to pH 5.0 the casein would again be precipitated, remain in the resin column, and again be redissolved by the elutriant acid. In addition, sugars and salts of the milk filtrate are carried through the resin. The varying quantities of these materials markedly influenced the diffusion current. However, with the method of standard addition the diffusion current was found to be strictly a linear function of the copper concentration. Although two polarograms must be recorded for each analysis, it is no serious time-consuming burden on the analyst—for example, with the instrument used in this work (Heyrovský micropolarograph, Model XIX) the two polarograms can be completed in 6 minutes.

The gross volume change between prepared sample and deproteinized and defatted milk filtrate due to change in total solids and fat elimination has a negligible effect on the concentration of trace components. Furthermore, the addition of perchloric acid compensates largely for this loss. This is well evidenced in Table I, which shows comparative data between spectrophotometric determinations of copper in fluid milk, powdered whole milk, and ice cream mix and their respective filtrates prepared in accordance with the proposed method. In all cases where spectrophotometric data are presented the mono-color dithizone procedure of Bendix and Grabenstetter (*2*) was employed. Seventy-five milliliters of filtrate were employed for all determinations made on filtrates. The aliquots were concentrated and digested with sulfuric and nitric acids. Five-gram samples of the powdered milk samples were prepared for analysis by acid digestion with sulfuric, nitric, and perchloric acids as described by Thompson (*9*) for preparation of samples for iron.

The necessity of buffering the filtrate is eliminated, as the pH need not be too critically controlled. Excellent copper recoveries may be obtained from filtrates ranging from pH 3.5 to 6.5. The neutral point of the ammonium hydroxide–perchloric acid system is pH 5.0, and the indicator titration described will with reasonable care yield a filtrate well within the pH limits. During the passage of neutralized filtrates (pH 3.5 to 6.5) through the resin, the pH will not vary appreciably from 1. Water washing of a prepared resin column will yield an effluent of pH 5 to 6. Thus, if the following conditions are adhered to, excellent copper recoveries will be obtained:

pH of prepared resin	5 to 6
pH during passage of filtrate	Approximately 1
pH at end of water wash	5 to 6

When the pH of the filtrate is greater than 7, the residual acid of the resin is rapidly depleted and the "copper-grabbing" properties are also depleted. If the pH is lower than 1, the copper will pass through the resin without absorbing.

Precision of the proposed method is demonstrated in Table II, which shows data obtained on a single sample of high copper content powdered whole milk, but using different volumes of filtrate and different sensitivities. Copper was added prior to the addition of the perchloric acid.

Table III shows comparative data obtained by the proposed method, the dithizone procedure, and a procedure in which the sample was first dry-ashed (ignited) and determined polarographically.

If polarographic equipment is not available, spectrophotometric methods may be employed. A suggested procedure is as follows:

Recover the elutriant acid and washings from the resin in a 300-ml. Kjeldahl flask. Concentrate by boiling. Cool and add 4 ml. of concentrated sulfuric acid and few milliliters of redistilled nitric acid. Complete destruction of organic matter in the usual

Table III. Comparison of Methods for Determining Copper

Sample	Spectrophotometric Dithizone Procedure, Sample Wet-Ashed	Polarographic Procedure, Sample Dry-Ashed	Proposed Method
	P.p.m.	*P.p.m.*	*P.p.m.*
Raw fluid milk	0.15	....	0.15
	0.15	....	0.20
Powdered whole milk[a]	.....	11.3	11.7
	.....	11.4	11.7
	.....	10.8	10.7
Duplicate determination	.....	....	10.8
Powdered whole milk[a]	15.2	....	15.0
Duplicate determination	15.1	....	15.5
	15.0	....	15.2
Powdered whole milk	0.80	....	0.80
	0.60	....	0.70
Ice cream mix	0.65	....	0.45
	0.20	....	0.35
Nonfat milk solids	.....	1.1	1.1
	.....	1.2	1.2

[a] Special high copper content samples.

manner, make the prepared solution up to 50 ml., and determine the copper in accordance with the method of Bendix and Grabenstetter (*2*).

In this manner a concentration of four times that possible by direct digestion of the sample is accomplished. Phosphates will have been eliminated as anions in passing through the resin the first time.

It is anticipated that the proposed method, or modifications thereof, will have wide application to the determination of traces of copper in a number of food products, particularly those foods which are in liquid form or can be readily dissolved or blended to a liquid form. In the latter case correction for volume changes will undoubtedly be of significance.

Investigations are now being undertaken in application of the concentration techniques described for the determination of traces of metals other than copper.

ACKNOWLEDGMENT

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Measuring the Distribution of Particle Size in Dispersed Systems

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An existing method for particle size measurement has been simplified. An apparatus and a technique are described which bring the investigation of particle size distribution and related research within the scope of laboratories having limited facilities. A consistent technique is suggested for testing all dispersions in general and concentrated emulsions in particular.

MOST of the methods now in use for particle size determination involve apparatus too complex and specialized to be feasible for the occasional needs of many investigators. A simplified procedure, useful for every system from concentrated emulsions to dilute suspensions and requiring only apparatus which can be assembled in any laboratory, would be advantageous.

Significant data on globule size distribution in emulsions having a high percentage of internal phase are difficult to obtain, since most methods used for particle size measurements make use of the laws of settling which do not apply under such conditions. Stoke's friction law, for instance, fails when the emulsifying agent itself constitutes a continuous phase within the emulsion, or when the external phase ceases to represent a homogeneous mass compared to the mean distance between internal phase globules.

A constant velocity of settling is assumed in most laws governing the fall of particles in a fluid medium. This condition can be obtained only in dilute systems. Addition of excess external phase until a dilution is reached, where it is known that the laws of settling do apply, may be the most straightforward way of handling concentrated emulsions.

The testing of all dispersed systems under the same conditions tends to increase the significance of the results. This is particularly evident when systems having nearly the same range of particle size but different concentrations are considered, for when these are diluted to a standard concentration for measurement, the probability of any particle suffering interference equivalent to a definite lengthening of its period of settling is approximately the same for each system.

In view of these considerations, the author has improved an existing micromanometric method (*2*, *3*, *5*, *6*, *9*) by simplifying calculations, minimizing work, and introducing a more consistent technique which extends the usefulness of the method.

THEORETICAL

Let

θ = angle inclined part of capillary manometer makes with horizontal
M = hypothetical membrane at juncture of manometer with sedimentation tube
A = cross-sectional area of sedimentation tube of uniform cross section
H = vertical distance from mid-point of M to surface in sedimentation tube
s = mean cross-sectional area of capillary manometer
d_i = density of internal phase of dispersed system
d_x = density of external phase (dispersion medium)
y = distance meniscus retreats in manometer during time t
w = grams of internal phase settling or rising past M during time t

A dispersed system is put into the large tube after the manometer has been filled with the dispersion medium (by filling the whole system with the dispersion medium, closing the stopcock, and pouring out the contents of the sedimentation tube). As particles of the internal phase move past the mid-point of M, the density of the system above this point decreases and the pressure which its weight exerts upon M diminishes, causing a receding of the meniscus in the manometer. As w grams of the dispersed phase settle or rise past M, a volume of dispersion medium weighing $w\frac{d_x}{d_i}$ grams is displaced upwards or downwards.

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The accompanying change in pressure, Δp_1, on the sedimentation tube side of M is given by

$$\Delta p_1 = -\frac{\left|w - w\frac{d_x}{d_i}\right|}{A} \quad (1)$$

To compensate this change, the meniscus falls in the manometer a distance y, producing a change in pressure, Δp_2, on the manometer side of M.

$$\Delta p_2 = -d_x X y \sin\theta \quad (2)$$

However, the fall in level in the manometer introduces into the main tube a quantity of dispersion medium. This amount of liquid added to the quantity in the tube increases the pressure on that side of M by Δp_3 which is given by

$$\Delta p_3 = \frac{sXyXd_x}{A} \quad (3)$$

Equating the pressures on both sides of M—i.e., letting $\Delta p_1 + \Delta p_3 = \Delta p_2$ and simplifying:

$$w = y\frac{d_x d_i}{|d_i - d_x|}(A \sin\theta + s) \quad (4)$$

Inspection of Equation 4 shows that, in order to measure the distribution of particle size in emulsions where ($|d_i - d_x|$) is small, the term ($A \sin\theta + s$) should have a value of about 0.05. Thus, for a 5% benzene in water emulsion the meniscus would retreat over 40 cm. in the manometer during a run on a 50-ml. sample. When testing dispersions of most solids, ($A \sin\theta$ + s) should approach 0.1 for an apparatus of the dimensions discussed below. Since y can be measured to the nearest 0.1 mm. with simple equipment, a precision of 1% is obtained on all but 2.5% or less of the dispersed phase.

The capillary should have an inside diameter of about 0.75 mm. with 8-mm. outside diameter for rigidity. The manometer gage should be about 50 cm. long. Sin θ should lie between 0.01 and 0.05. The diameter of the main tube should not exceed 16 mm. and it should be long enough to contain 50 ml. below and above M. It is not wise to use a capillary with much smaller bore than that mentioned, for capillarity and lack of uniformity over any useful length would introduce errors. The influences of temperature variations, inertia, evaporation, and contamination of the walls of the manometer contribute to make 1% the limiting accuracy for this method. However, excepting carefully controlled conditions, the errors which arise in most applications of the equations governing fluid friction do not permit greater accuracy.

If we let P denote the percentage of the total mass of internal phase settling or rising past M during time t, while W represents the total mass of internal phase in the original system

$$P = \frac{w}{W} \times 100 \quad (5)$$

If Y represents the total recession of the meniscus in the manometer during a run, it follows from Equation 4 that

$$P = \frac{y}{Y} \times 100 \quad (6)$$

When a run is made, the position of the meniscus as read on a scale strapped under the manometer must be recorded at measured time intervals. Also, H must be known to the nearest millimeter. Equation 6 is used to compute values of P corresponding to observed values of y.

The actual amount of material represented by any value of P is a heterogeneous system composed of (a) particles constituting size groups the whole of which have passed M during the associated time elapsed, and (b) smaller particles belonging to all the other size groups present in the polydispersed system. The rate of sedimentation (up or down) of all these classes together is dP/dt. Thus, $t \times dP/dt$ gives the percentage of dispersed phase in these latter size ranges. Therefore, if S denotes the percentage of internal phase in size ranges which have, in their entirety, passed M during time t

$$P = S + (t \times dP/dt) \quad (7)$$

If P is plotted against t, the ordinate intercept on the P axis of any tangent to the resulting curve will be a value of S (*1*, *4*, *7*, *8*). Thus, the difference between the ordinate intercepts of any two tangents to this P,t curve gives the percentage, ΔS, of the total dispersed phase corresponding to the size range $r \ldots r_n$ calculated from Stoke's law for the time interval between the abscissas at these points of tangency. The distribution of particle size can now be tabulated for all the material in the dispersed phase.

The following form of Stoke's law should be used in this calculation:

$$r^2 = \frac{9 \times \eta \times H}{2 \times D \times g \times t}$$

where

r = radius of particles moving with constant velocity
η = viscosity of dispersion medium, poise
D = absolute density of internal phase, $|d_i - d_x|$
g = acceleration of gravity
t = time of sedimentation, seconds

In the author's experience, most emulsions having a percentage of internal phase not greater than the close-packing ratio for spheres of uniform size (74.048%) do not break upon dilution with excess of their pure external phase. In true emulsions the protecting films of emulsifying agent (no matter whether they are composed of solid particles, polar molecules, electrically charged ions, etc.) surrounding a globule of internal phase can exist unchanged completely apart from any other such films on any other globule. The same characteristics of the protecting layer which prevent globules from coalescing when they are almost in contact with one another will function equally well when the same globules collide in a diluted emulsion.

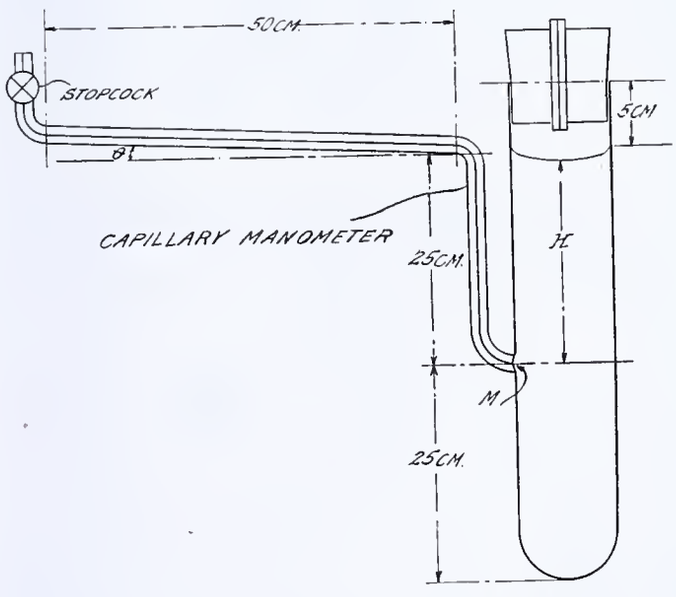

Figure 1. Sedimentation Apparatus

Emulsions which break upon dilution are generally those having more than 74.048% of internal phase, and usually, these are partially pseudo-emulsions—that is, they contain internal phase globules which are not covered by emulsifying agent but are pocketed mechanically among protected globules. Upon dilution these unprotected globules coalesce to form a layer of free internal phase which destroys the whole emulsion. When the close-packing ratio is exceeded, the films of emulsifying agent are in continuous contact over large areas of their surfaces and often become actually a separate continuous phase. In such systems, dilution destroys the continuity of the network of emulsifying agent and such emulsions often break spontaneously.

However, for most emulsions having concentrations of internal phase between 20 and 74%, dilution may be the only practical means by which they can be reduced to a form where the dis-

tribution of particle size can be measured. Furthermore, the cases other than the exceptions discussed above give little evidence that dilution will influence the size of dispersed particles.

EXPERIMENTAL

The dimensions of the apparatus have been discussed and are repeated in Figure 1. The capillary is selected for its uniformity by measuring the length of a piece of clean mercury at various portions of the length of the tube to be used as the manometer. Available in most laboratories is a room or bath where a fixed temperature can be maintained to within ±0.5° C., which is sufficient even when liquids having high coefficients of expansion are used in this apparatus. No calibration of the apparatus is necessary.

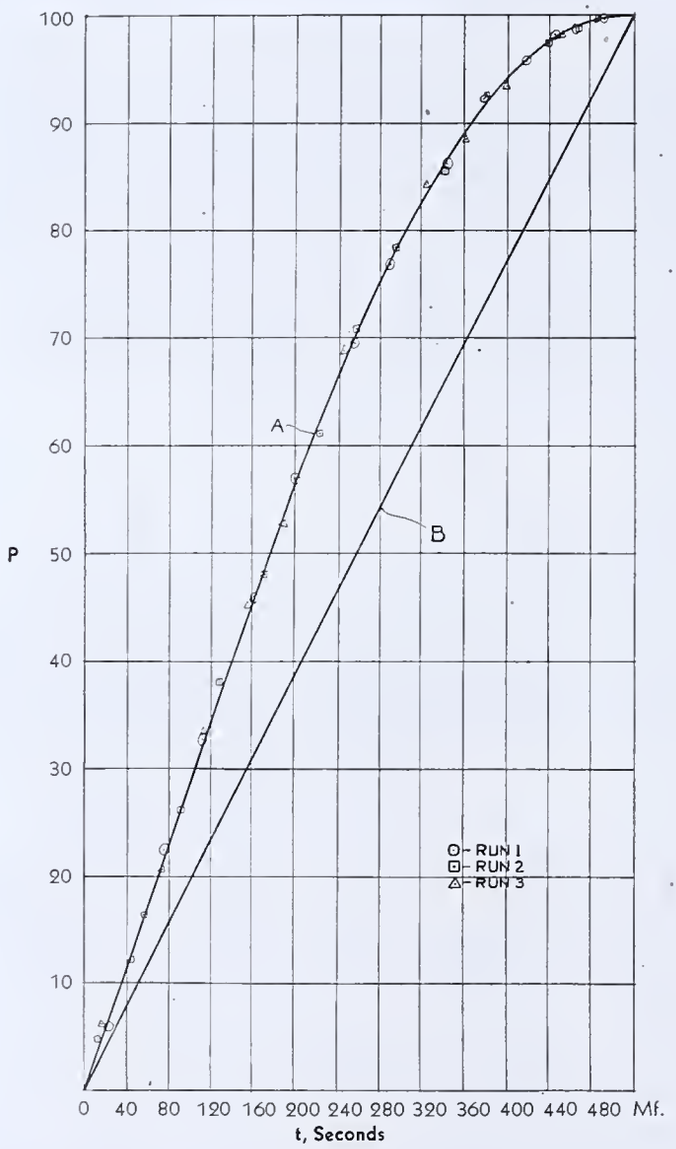

Figure 2

If any difficulty arises in obtaining the desired sin θ, a trial run can be made, during which the positions of the clamps are adjusted to bring the manometer as close to the horizontal as necessary to obtain the desired accuracy. With the manometer full of dispersion medium and the main tube empty, a run is begun by pouring a dispersion into the main tube to a predetermined mark where the meniscus in the manometer is supported near the beginning of the manometer scale close to the stopcock. The timer is started immediately and, simultaneously, the stopcock is opened. A few readings must be taken at close intervals, so that the position of the meniscus at zero time can be accurately obtained by a short extrapolation. The rate of recession of the dispersion medium in the manometer due to evaporation via the stopcock and in the main tube should be determined for each liquid used. Volatile liquids will give a recession of only a fraction of a millimeter per hour. Correction can be made for this on the finest dispersions. Normally, readings should be stopped when the rate of recession approaches this value.

Table I. Particle Size Distribution

$r \times 10^4$ cm.	ΔS (%)
> 10.1	2.9
10.1 – 8.97	5.4
8.97 – 8.24	5.6
8.24 – 7.74	7.6
7.74 – 7.51	5.4
7.51 – 7.16	4.3
7.16 – 6.74	12.4
6.74 – 6.42	16.5
6.42 – 6.14	14.7
< 6.14	25.2

Curve A in Figure 2 shows the mean P,t curve for three runs on identical suspensions of 1.80% Fe_3O_4 in 1,4-dioxane (diethylene dioxide). The density and viscosity at 25° of the p-dioxane were 1.03 grams per ml. and 1.20 cp., respectively. The ordinate deviations of the points from the curve are 1% or less in all but the initial values. Results which are reproducible to within 1% for such coarse systems show that higher precision is possible when dispersions having longer settling times are tested. Curve B, included for comparison, represents a monodispersed system composed of only the smallest particles in A. Table I gives the weight percentages of dispersed phase corresponding to various size ranges.

This method is suggested for measurements upon emulsions of all concentrations at various ages to determine the effects of aging upon the distribution of globule size. Samples of creams of such emulsions may be diluted with dispersion medium to a concentration, for example, of approximately 5% internal phase as the standard form to be tested. While some doubt may exist as to whether or not such dilution affects the distribution of globule size, there is no doubt that, for a series of similar emulsions all tested under approximately the same conditions, significant results for comparison may be obtained. Systems of mixed pigments where photographic or colorimetric methods give dubious results are readily tested with this apparatus.

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Collective Index to Analytical Edition

Type of the Collective Index to the ANALYTICAL EDITION has been held since the second printing of the book several months ago, but the present need for type metal is such that it cannot be held much longer. Any who want to secure copies are advised to send orders now to the AMERICAN CHEMICAL SOCIETY, 1155 Sixteenth St., N. W., Washington 6, D. C.

NOTES ON ANALYTICAL PROCEDURES

High-Speed Filter Aid for Chromatographic Analysis

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SINCE a specially prepared heat-treated siliceous earth (Hyflo Super-Cel) sold by Johns-Manville was first recommended by Strain (*5*) to preserve the advantages inherent in the use of finely divided adsorbents in chromatographic analysis and to increase the rate of flow of liquid through the adsorbent column, it has found wide application for these purposes. It may be used with various adsorbents in addition to that originally recommended (*4*).

At the time Hyflo Super-Cel was first recommended by Strain, this product allowed the highest rate of flow of liquids through a column of any of the filter aids commercially available. Several years ago, however, several filter aids became available which allow higher rates of flow. Nevertheless, Hyflo Super-Cel continues to be the filter aid in general use for chromatographic analysis, as shown by recent articles (*1*, *2*, *3*, *7*, *8*). Celite No. 545 allows the highest rate of flow of any filter aids presently available. It is white in color and therefore suitable for use as a filter aid and diluent in chromatographic analysis. Table I shows the rates of flow resulting from using Celite No. 545 in place of Hyflo Super-Cel in adsorption columns containing some typical adsorbents mixed with filter aids.

The magnesia used in the tests was Micron Brand No. 2641, made by Westvaco Chlorine Products Co., Newark, Calif., which is widely used for chromatographic analysis. The materials were weighed in the proportions listed and thoroughly mixed by passing through a kitchen-type flour sifter three or four times. In all cases the adsorption columns were 3.3 cm. in diameter. The adsorption columns were filled with adsorbent mixture to the height listed in Table I, and fitted to modified side-arm separatory funnels. A battery of six modified side-arm separatory funnels was connected to a water pump capable of developing a maximum vacuum of 2 cm. of mercury, and the solvents were drawn through the columns under the full force of the pump. The solvents were run concurrently through comparable adsorbent columns using Hyflo Super-Cel and Celite No. 545. All determinations were in triplicate, and the averages of three determinations are shown in Table I.

The data in Table I show that substituting Celite No. 545 for Hyflo Super-Cel is a diluent or filter aid in chromatographic adsorption analysis will result in greatly increased flow rates through the adsorbent columns. The saving in time can be appreciable when large numbers of chromatographic analyses are made during a day, or when large volumes of solvent must be passed through an adsorption column.

Table I. Rates of Flow of Solvents through Adsorbent Columns

Adsorbent	Solvent	Column Height	Time for Solvent to Pass through Column: First drop	Time for Solvent to Pass through Column: 100 ml. after first drop
		Cm.	*Min.*	*Min.*
1 part Super-Cel to 1 part MgO	Petroleum ether	10	3.5	9.5
1 part Celite 545 to 1 part MgO	Petroleum ether	10	2.5	6
3 parts Super-Cel to 1 part MgO	Petroleum ether	15	4.25	6.25
3 parts Celite 545 to 1 part MgO	Petroleum ether	15	1.25	2.25
1 part Super-Cel to 1 part fuller's earth	Water	7.5	4.5	29
1 part Celite 545 to 1 part fuller's earth	Water	7.5	2.25	12

These heat-treated siliceous earths will adsorb chlorophylls and carotenols (*6*), but Celite No. 545 is less strongly adsorbent than Hyflo Super-Cel for these pigments, as shown by the greater width of pigment bands on the Celite No. 545 columns when equal amounts of pigments are adsorbed on the two siliceous earths. Some lots of Hyflo Super-Cel slightly adsorb carotenes, but not sufficiently to prevent petroleum ether from readily washing the carotenes through any column of the sizes normally used. Adsorption of carotene by Celite No. 545 has not been observed.

To test the loss of carotenes upon passage of a solution of carotenes through the two siliceous earths, aliquots of a solution of carotenes in low-boiling petroleum ether (boiling range 30° to 60° C.) were run through adsorption columns of Hyflo Super-Cel and Celite No. 545. The columns were 3.3 cm. in diameter and were filled to a height of 25 cm. with the siliceous earths. The aliquots were diluted to 75 to 100 ml. with petroleum ether, the solutions were poured on to the dry columns which were under suction, the carotenes were washed through the columns with fresh petroleum ether, and the solutions were made up to a volume of 200 ml. The total carotenes in the original solutions and in the solutions which had passed through the columns were then determined in a photoelectric colorimeter. Results are shown in Table II.

Table II. Recovery of Carotene from Hyflo Super-Cel and Celite No. 545 Columns

Carotene Added	Carotene Recovered: Celite No. 545	Carotene Recovered: Hyflo Super-Cel
Micrograms	*Micrograms*	*Micrograms*
402	407	402
207	207	201
102	100	100
89	89	83
39	39	37
14	14	14

The minimum amount of carotene that could be definitely determined in the colorimeter at the concentrations involved avried from 2 micrograms for the most dilute solution to 5 micrograms for the most concentrated solution. Within these limits, no significant loss of carotenes could be detected when the carotene solutions were passed through the Celite No. 545 columns. A small but significant loss of carotenes occurred in some instances with the Hyflo Super-Cel columns.

Thompson *et al.* (*7*) report that a contaminant absorbing light in the region of 310 to 370 millimicrons dissolved from a mixture of calcium hydroxide and Hyflo Super-Cel with petroleum ether and benzene mixtures. It has been observed in this laboratory that passing methanol or water through a column of Hyflo Super-Cel or Celite No. 545 will wash out a yellow pigment. Less of this pigment has been obtained from Celite No. 545 than from equal volumes of Hyflo Super-Cel in the samples tested, but sufficient data are not available to determine if this is a general relationship. This pigment will cause a marked light absorption on a photoelectric colorimeter using a Corning No. 554 (Signal Blue) glass color filter as in the colorimetric determination of riboflavin. This pigment will not normally interfere with the determination of riboflavin if riboflavin is first adsorbed on a column of mixed

Table III. Recovery of Riboflavin from Hyflo Super-Cel and Celite No. 545 Columns

Riboflavin Added	Riboflavin Recovered	
	Hyflo Super-Cel	Celite No. 545
Micrograms/ml.	*Micrograms/ml.*	*Micrograms/ml.*
2.8	2.5	2.9
5.5	5.3	5.4
9.8	9.3	9.6
15.0	14.7	14.8
19.8	19.8	19.6

adsorbent and siliceous earth and then eluted, as the pigment is normally discarded with the first washings from the adsorption column. Celite No. 545 is, however, preferable to Hyflo Super-Cel in the preparation of adsorbent columns for chromatographic analysis. Although riboflavin does not form adsorption bands on columns of these siliceous earths, a small amount of riboflavin appears to be lost upon passage through these materials. This amount is generally not significant for Celite No. 545, but may be significant for Hyflo Super-Cel.

To determine the loss of riboflavin in Hyflo Super-Cel and Celite No. 545, columns 3.3 cm. in diameter were filled to a height of 12 cm. with these materials. A solution was made of synthetic U.S.P. riboflavin in water containing 5 ml. of glacial acetic acid to the liter. Aliquots were taken of this solution for determination of the riboflavin content in a photoelectric colorimeter. Similar aliquots were poured on the dry columns, the solutions drawn through the columns under suction, and the columns washed free of pigment with water containing acetic acid. The riboflavin solutions were diluted to a volume of 100 ml., and the riboflavin content was determined in the photoelectric colorimeter. The results were corrected for the interfering pigment which equaled 1.40 micrograms per ml. of riboflavin for Hyflo Super-Cel and 0.54 microgram per ml. of riboflavin for Celite No. 545. These results are shown in Table III.

The concentration of riboflavin that could be definitely determined varied from 0.1 microgram per ml. for the most dilute solution to 0.3 microgram per ml. for the most concentrated solution tested. The loss of riboflavin in the Celite No. 545 column is, therefore, barely significant. An appreciable loss of riboflavin was experienced upon passage of the solutions through the Hyflo Super-Cel columns.

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Determination of Hydrogen in Highly Fluorinated Carbon Compounds

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A PROCEDURE for analyzing compounds of the type C_nF_m for carbon and fluorine has been previously described (*2*). A method is described in this paper for the determination of hydrogen in similar highly fluorinated carbon compounds. The method consists of decomposing the sample with magnesium in an evacuated quartz tube at about 700° C., passing the residual hydrogen over hot copper oxide, and absorbing the water formed in a weighed phosphorus pentoxide tube.

Mention has been made (*1*) of an experiment in which the hydrogen content of fluoroform was determined by repeatedly passing the sample over heated sodium metal until it was completely decomposed and then measuring the volume of the residual hydrogen. Attempts were made in this work to use sodium or potassium in the decomposition, but the results were erratic and consistently low. While the temperature in the hottest portion of the tube was sufficiently high to decompose sodium or potassium hydrides, it is believed that combination of the metal and hydrogen took place in cooler portions of the tube. When magnesium was used no difficulties of this nature were encountered.

The apparatus, Figure 1, consists essentially of alternate sample bulbs, *C* and *D*, a quartz tube, *G*, in which the nickel boat containing the magnesium is placed, a copper oxide container, *J*, the absorption tube, *I*, which contains phosphorus pentoxide, and an automatic gas pump, *H* (*3*) to recycle unreacted hydrogen and sample. *G* is heated by a two-piece resistance furnace which can be removed without disconnecting the tube. *J* is wound with a Nichrome coil which serves as a heating element.

Experimental Procedure. The sample, in the case of substances boiling below about 50° C., was weighed by introduction into the previously evacuated and weighed bulb, *D*. For higher boiling substances the sample was weighed directly into a small glass cup which was then placed in tube *C;* it was necessary to freeze and degas these samples before analysis. The samples were of such size that between 30 and 40 mg. of water would be formed.

The nickel boat, filled with approximately 1 gram of magnesium turnings, is introduced into the quartz tube and the system sealed and evacuated. The furnace, previously heated to 650° to 700° C., is lowered and clamped about the quartz tube. The copper oxide is heated to about 300° C. Evacuation is continued until no further gas is given off by the magnesium. The sample is then admitted to the magnesium and the circulating

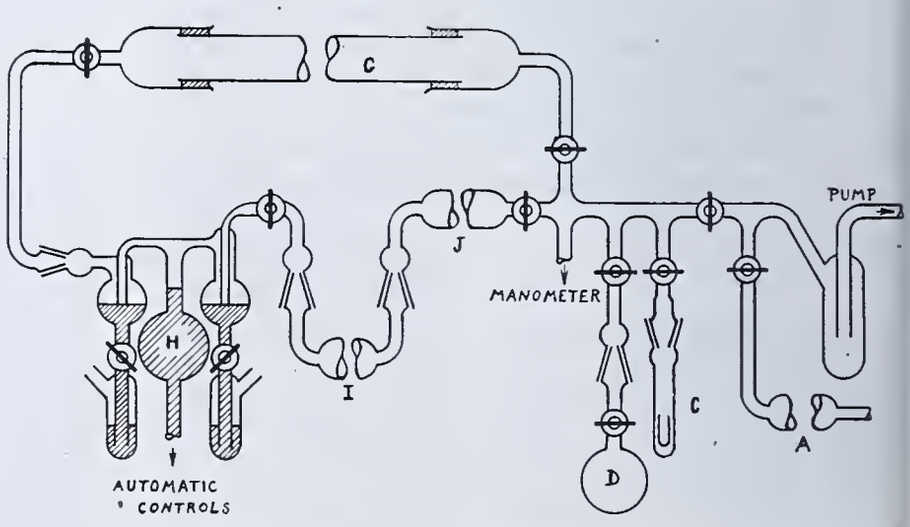

Figure 1. Apparatus

pump started. Pumping is continued until the pressure falls to approximately 3 mm., the lower operating limit of the pump. By withdrawal of the mercury from the valves, the residual gas trapped in the pump is released to the combustion system.

When the pressure has fallen to essentially zero, dry air is admitted through tube *A* which contains phosphorus pentoxide. The absorber tube, *I*, is then removed, protecting ground-glass caps are placed over the ends, and the tube is reweighed. The amount of hydrogen present in the original sample can then be readily calculated.

The procedure was checked by analysis of methyl fluoride and tank hydrogen. Calculated for CH_3F: H, 8.89. Found: H, 8.51. Calculated for H_2: 0.357 gram of H_2O. Found: 0.0345, 0.0336 gram of H_2O.

Three of the compounds analyzed are presented as examples.

Compound	Hydrogen Calculated	Found
C_3F_7H	0.59	0.55
$C_3F_6H_2$	1.32	1.29
C_3F_6HBr	0.43	0.40

This work was done under the sponsorship of Minnesota Mining and Manufacturing Company.

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Electronic Make and Break for Relay Operation

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IN THE operation of a constant-temperature air bath where mechanical circulation was not permissible and convection provided the only stirring, the problem of supplying heat in small increments to prevent overregulation of the bath temperature was met. If the heat source remained "on" continuously, enough heat to raise the bath some 2° or 3° had been supplied by the time the thermoregulator responded to the temperature increase. By the make and break arrangement heat was supplied for 3 to 4 seconds only of each 30 seconds, to allow dissipation by convection, and regulation to ±0.2° was obtained.

The first device employed to effect this was a clock with a sweep second hand brushing over a metallic strip, so that an electrical contact was maintained for 3 to 4 seconds in each 30 seconds. A very reliable apparatus is described by Booth and Martin (*1*). It was thought that these mechanical devices might be simplified by the use of an adaptation of the relaxation oscillator. However, the necessity for the auxiliary power supply equipment was undesirable and a modification of the Serfass regulator (*3*), employing one electron tube and a few resistors and condensers, was chosen as the simplest solution. The principle of a pickup and dropout action has been described by Henney (*2*).

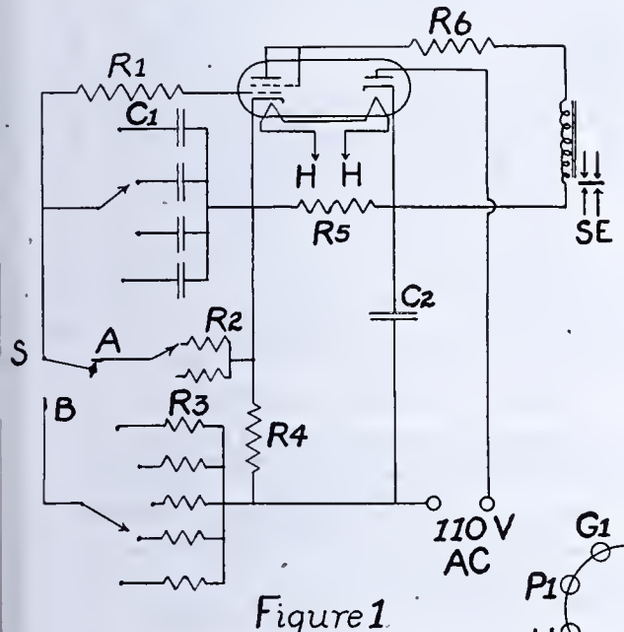

Figure 1

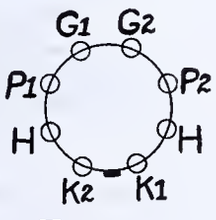

Figure 2

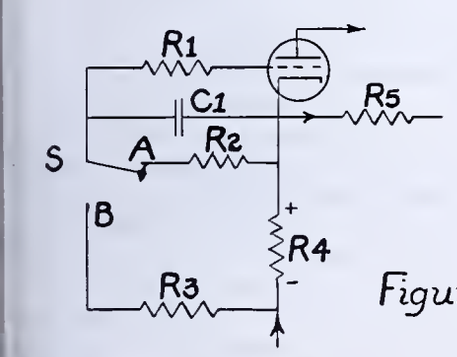

Figure 3

Resistor and Condensers

R_1. 1 meg. 0.5 watt
R_2. 1 meg. 0.5 watt
20 meg. 0.5 watt
R_3. 1 meg. 0.5 watt
2 meg. 0.5 watt
5 meg. 0.5 watt
10 meg. 0.5 watt
20 meg. 0.5 watt
R_4. 1000 ohms 5 watts
R_5. 15,000 ohms 2 watts
R_6. 10,000 ohms 2 watts
C_1. 0.1 mfd. 250 volts
0.25 mfd. 250 volts
1.0 mfd. 250 volts
4 mfd. 250 volts
These are paper condensers.
C_2. 8-mfd. 250-volt dry electrolytic condenser

The basic circuit is that of Serfass, modified by a condenser to introduce time delay into the rise and fall of the grid voltage, with a mechanical device for opening and closing the grid circuit. This latter was accomplished by using a double-pole double-throw relay switch, one pole of which acted as a make and break in the grid circuit, *S*, and the other pole as an off-on switch for outside auxiliary apparatus, *E*. Switch *S*, appearing in the grid circuit of Figures 1 and 3, and switch *E* of Figure 1, are the two halves of the double-pole double-throw relay contacts. Current in the relay coil activates switches *E* and *S* simultaneously.

Figure 3 is a simplified version of the grid circuit. Electrons flow up through R_4 to the cathode of the tube. Voltage is maintained across resistance R_4 by the by-pass resistor, R_5. If section *S* of the double-pole double-throw switch is in position *B* electrons will flow through R_3 to charge condenser C_1. Initially *S* is in position *A*, and the grid is, in effect, short-circuited to cathode by R_1 and R_2. Current flows in the tube and causes the relay to close, switching *S* from *A* to *B* positions. Electrons flow through R_3, charging C_1 so that the grid side becomes negative. As C_1 charges the plate current of the tube decreases, and when it has reached a value too weak to hold the relay closed, *S* is switched from *B* to *A*. The time delay before the relay opens is controlled by the capacity of C_1 and the value of resistor R_3.

C_1 can now discharge through R_2 and does so at a rate controlled by the condenser capacity and the resistance of R_2. As C_1 discharges, the grid potential approaches that of the cathode and the plate current rises. At a value of plate current sufficient to close the relay, *S* is switched from *A* to *B* again and the cycle repeats. The time of charge and discharge is regulated by condenser C_1. The ratio of off to on periods can be varied by changing the ratio of the values of R_3 and R_2.

When R_2 and R_3 are equal the relay will be open and closed roughly for equal periods of time. If R_3 is greater than R_2 the time for charge of C_1 will be greater than for discharge and the relay contacts will be closed for a longer period than they are open. If the power fails the contacts open. When R_2 is greater

than R_3 the relay contacts are open for a longer time than they are closed. In order to vary the off-on ratio, a number of resistors from 1 to 20 megohms on a multiple contact switch were substituted for R_3. In order that the period of time for which the contacts were closed might be either greater or less than the time period they were open, a single-pole double-throw switch was used for R_2 with two resistors of 1 and 20 megohms. Changing from one resistor to the other in R_2 inverts the open-closed time ratio.

The complete circuit is shown in Figure 1, and the tube base (117L7/M7 GT) is shown in Figure 2. The relay is a double-pole double-throw Guardian (Guardian Series 5, D.C. coil 7.5 ma., 800 ohms). A 10,000-ohm resistor, R_e, was put in the plate circuit to reduce the plate current to fit the relay. This resistor should be adjusted to the relay coil used. The tension of the relay spring had to be increased to get reliable operation on the pullout action. The pullout action may manifest a certain vibration or chattering due to the half-wave rectification of the vacuum tube, but this has not affected the operation of the device. The rapidity of the cycles is governed only by the response of the relay.

This apparatus has been in operation for some months and has functioned quite satisfactorily. The device may also be used for operation of an enclosed magnetic stirrer where the cycle or rate of stirring might be varied over wide limits, or any apparatus where electrical impulses are required periodically.

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Automatic Cutoff for Electrically Heated Water Still

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AN ELECTRICALLY heated glass water still equipped with the constant-level device described by Holmes (*1*) has been installed in this laboratory to furnish redistilled water for microchemical and other analytical work. The still is entirely automatic and requires no attention except for periodic removal of sediment in the still pot and refilling of the water reservoir. Since it is frequently desirable to allow the still to run without attention, a means has been devised for automatically shutting off the heater when the reservoir is empty, to prevent breakage of the distillation flask. This device is simply constructed and positive in action.

A ⅋ 14/20 outer joint was sealed onto the standpipe about 60 mm. above the constriction (7 mm. in inside diameter) as shown in Figure 1. A mercury thermoregulator was sealed through a ⅋ 14/20 inner joint, so that its bulb extended as nearly as practical across the standpipe. The thermoregulator was made from 6-mm. capillary tubing having a bore of approximately 0.5 mm. An expansion chamber equivalent to at least 25° C. must be left at the top of the regulator.

Equally good results were obtained with an 11-mm. glass side tube on the standpipe in place of the ⅋ 14/20 outer joint, and with a regulator, also without ⅋ joint, held in place by a short sleeve of rubber tubing. Small thermoregulators, both fixed- and variable-setting, are commercially available in the right-angle style.

The circuit (Figure 2) consists essentially of a two-pole normally open relay, R; one pole and the holding coil are in series with the mercury thermoregulator, T, in the secondary circuit of transformer TR, and the other pole is in series with the line switch, S_1. S_2 is used to by-pass the thermoregulator until the water in the standpipe is warm enough to close the thermoregulator contacts and also to operate the pilot lights, P_1 and P_2, which indicate whether or not the heater, H, is under the control of the thermoregulator. S_3, a momentary contact switch, serves as a reset for the relay; one pole completes the 115-volt circuit and the other completes the secondary circuit. Capacitors C_1 and C_2 are placed across the mercury thermoregulator and the relay contacts to reduce sparking when contact is made or broken.

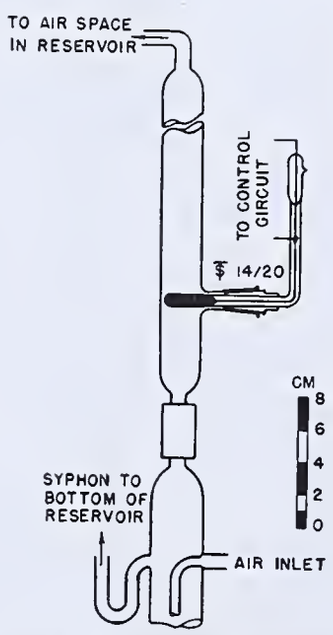

Figure 1. Standpipe and Mercury Thermoregulator

The air above the liquid in the standpipe is much cooler than the liquid. This difference in temperature as the liquid level drops below the thermoregulator bulb serves to open the regulator contacts and turn off the heater. The proper operating temperature for the thermoregulator can be easily determined by substituting a thermometer for the thermoregulator in the standpipe and observing the temperature as distillation progresses. In the unit now in use the regulator is set to open at 50° C. This temperature is, however, somewhat dependent on the size and shape of the constriction and on the rate of distillation.

The same regulator setting has been used satisfactorily with reservoirs having capacities of 1, 10, and 20 liters. The bottom of the reservoir should not be lower than the constriction in the standpipe. The reservoir is then completely empty when the heater is turned off.

To operate the still, line switch S_1 is closed, by-pass switch S_2 is thrown to position 1, and reset switch S_3 is closed momentarily. The heater is now on and pilot light P_1 burns to serve as a warn-

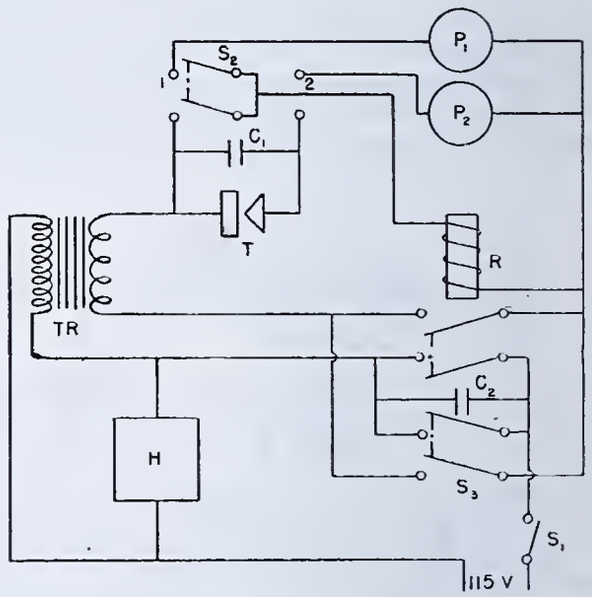

Figure 2. Automatic Cutoff Circuit

S_1. Line switch, SPST toggle switch, 3 amperes at 250 volts
S_2. By-pass switch, DPDT toggle switch, 1 ampere at 250 volts
S_3. Reset switch, DPST, momentary contact switch, normally open, 12 amperes at 125 volts
R. Relay, DPST, normally open, 6-volts, 60 cycles, 8 amperes at 110 volts, Potter and Brumfield type MRA-4
TR. Transformer, signal, 4 volts, United Transformer Co. Type SC-3
T. Mercury thermoregulator
P_1, P_2. Pilot lights, 6 to 8 volts, Mazda No. 46
H. Heater, 550 watts, Precision Scientific Co. Type H
C_1, C_2. Capacitors, 0.1-mfd., Cornell-Dubilier Type DT-4P1

ing that the heater is not under the control of the regulator. After the water has warmed sufficiently to close the regulator contacts (20 to 30 minutes with the unit now in use), the by-pass switch is thrown to position 2 and S_3 is closed momentarily. Pilot light P_2 now burns to show that the heater is controlled by the regulator. The temperature drop due to the change from liquid to gas phase at the thermoregulator bulb as the reservoir empties causes the regulator contacts to open. This break in the secondary circuit immediately opens the relay and shuts off the heater and primary coil of the transformer; thus, continued arcing at the regulator points and relay chatter, which are common when alternating current is used with mercury regulators, are eliminated. It is not possible to turn the heater on again until the relay is reset, either manually or electrically.

The control system can be somewhat simplified as follows: The primary coil of the transformer can be wired directly to the line switch, S_1, instead of to the relay as shown in Figure 2. (The heater must, of course, remain wired to the relay as shown in Figure 2.) The primary coil of the transformer is then energized whenever S_1 is closed. S_3 can then be replaced by a doorbell-type push button in parallel with the secondary pole of the relay. Signal transformers are designed for continuous duty and it does no harm if the primary of the transformer remains energized after the heater is turned off.

S_2 can be a shorting switch ("make before break") instead of one of the common toggle type. Such a switch would eliminate the necessity for resetting the relay after changing the position of S_2.

The signal lights can be eliminated. The thermoregulator is then connected directly to the coil of the relay and a single-pole single-throw toggle switch is placed in parallel with the thermoregulator. The switch is "on" during the preliminary warm-up and "off" after contact is made at the thermoregulator.

The entire cost of materials listed under Figure 2, exclusive of the thermoregulator and the heater, is about $9. These makes, types, and capacities were used because they were immediately available. Other materials of similar characteristics could have been used.

This device should find use in other applications where change in level of a liquid results in lowered temperature.

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A Reaction of Lead Soaps with Sodium Iodide

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PREVIOUS investigators (*1*, *4–7*) have shown that reactions of the double-decomposition type can take place in insulating solvents such as dry benzene between heavy metal soaps and dry hydrogen chloride or metallic chlorides, such as stannic chloride, which are also soluble in benzene.

The present communication summarizes preliminary work which has been done to apply a reaction of lead soap with sodium iodide to the development of a suitable method for determining free organic acids in lead soaps. The essence of an analytical method is presented, and semiquantitative data, obtained on lead soaps made from organic acids of differing molecular weights, are given.

In attempting to develop a method for the determination of free organic acids in lead soap it was found that the addition of an acetone solution of sodium iodide to an acetone-benzene solution of lead soap apparently causes a double-decomposition reaction as follows:

$$\text{Lead soap} + \text{NaI} \longrightarrow \text{a lead iodide} + \text{sodium soap}$$

The mixture turns a bright greenish yellow upon addition of the sodium iodide solution and flocs of soapy-looking material separate out when the solvents are evaporated to dryness. The residue after evaporation is bright yellow, and gives an opalescent, frothy, soapy extract with water, which is alkaline to phenolphthalein and gives a curdy precipitate with barium chloride solution (*3*). No lead was found in the benzene extracts from five dried residues extracted individually. This indicates that all the benzene-soluble lead soap had been converted to a benzene-insoluble lead iodide, possibly a basic iodide.

Both commercial lead oleate and lead naphthenates have been treated in solution with sodium iodide with the same general results, the sodium oleate formed separating out more quickly upon solvent evaporation than the sodium naphthenate—i.e., seeming to be more insoluble in these solvents, as would be expected.

Warm 95% acetone (5% water) is an excellent solvent for extracting organic acids, including oxidized fatty acids, from lead soap or lead soap lubricants. However, a small amount of lead soap is dissolved concurrently, which invalidates the usual titration for free organic acids of the combined acetone extracts, because the heavy metal soap is titrated as free acidity (*2*).

Some work has been done in using the foregoing lead soap–sodium iodide reaction to transform such dissolved lead soap in combined acetone extracts to sodium soap and a lead iodide. By extracting the dried residue left after evaporation of the acetone with hot benzene it is possible to isolate the free organic acids (together with a small amount of sodium soap), leaving behind the lead iodide, excess sodium iodide, and most of the sodium soap. The extracted material taken up in alcohol can then be titrated for free organic acids in the usual manner, as the sodium soap present does not interfere.

Semiquantitative results for free acids on lead naphthenates made from naphthenic acids of different molecular weights, using the above technique, indicate a much higher free acid content for the soap made from high molecular weight acids, the following comparative figures being obtained:

Lead Soap	% Organically Combined Pb	% Free Fatty Acids as Oleic
From 176 saponification value acids (318 calculated molecular weight)	18.5	10
From 282 saponification value acids (199 calculated molecular weight)	41.3	3

The greater reactivity of the lower molecular weight acids with lead may account for the lower free acidity in this soap.

Indications have also been obtained that sodium iodide in acetone solution does not react with lead oxide (which may be present in lead soaps) to give free caustic alkali, which would, of course, invalidate the determination of free acidity. However, this reaction does occur when lead oxide is heated with aqueous sodium iodide solution.

It is proposed for the future to ascertain by analysis whether the lead iodide compound formed is a basic salt or the normal diiodide, and thus gain some idea of the structure of the dissolved lead soap. It is also proposed to complete work on the analysis of lead soaps containing weighed additions of free organic acids, using the technique described above, in order to define the accuracy and reproducibility of this method as a means of determining free organic acids in lead soaps.

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New Standard for Use in Ultimate Analysis of Organic Compounds Especially Suited for Microprocedures

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FOR the past three years *S*-benzylthiuronium chloride (benzyl-isothiourea hydrochloride, listed by Eastman Kodak Company in Catalog 34 of Organic Chemicals) has been used in this laboratory as a standard material in the ultimate analysis of organic compounds. It more nearly fulfills the criteria established by Hillebrand (*2*) for judging the fitness of material used as a standard to check on analytical procedures than most of the standards in common use.

Since *S*-benzylthiuronium chloride contains the five elements most commonly encountered in organic analysis—namely, carbon, hydrogen, nitrogen, chlorine, and sulfur—and since it also contains a benzene ring, a thio ether linkage, amine and imine groups, and an ionizable chlorine atom, it is suitable for checking the methods of analysis of any or all of the five elements in a wide variety of compounds. Its use as a standard for carbon and hydrogen not only serves to prove that combustion is complete but also shows the effectiveness of the removal of the acid-forming gases of the three elements most likely to interfere with this determination.

The synthesis and recrystallization of the pure compound (*1*) require no unusual apparatus or techniques and its purity is readily established by confirming analyses for any two or more of the five elements. After drying at 110° C., no precautions are required for its storage, since under ordinary conditions it is stable and nonhygroscopic.

Table I. Analysis of *S*-Benzylthiuronium Chloride

Element	Methods	Percentage Composition Found (Range)	Theory
Carbon	Dry combustion	47.22–47.55	47.40
Hydrogen	Dry combustion	5.36– 5.53	5.47
Sulfur	Catalytic combustion and peroxide bomb	15.71–15.89	15.82
Chlorine	Catalytic combustion	17.41–17.59	17.49
Nitrogen	Dumas and Kjeldahl	13.68–13.88	13.82

The standard has been analyzed repeatedly for the different elements by the following procedures: dry combustion carbon and hydrogen, catalytic combustion and peroxide bomb sulfur, catalytic combustion chlorine, and Dumas and Kjeldahl nitrogen. Representative analytical data obtained on the compound are shown in Table I.

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PRESENTED at the Spring Meeting of the Philadelphia Section, AMERICAN CHEMICAL SOCIETY, June 13, 1945.

Determination of Sulfur in Sulfur Compounds with Benzidine Hydrochloride

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IN ORDER to determine total sulfur by the benzidine method in waters containing bisulfites, sulfites, hyposulfites, and thiosulfates, it was necessary to oxidize these compounds to simple sulfates previous to adding the benzidine hydrochloride reagent.

Oxidizing agents such as alkaline potassium permanganate (*15*), the Parr oxygen bomb (*1, 19*), or some of the strong oxidizing methods developed for blood, plasma, or urine (*2, 7, 10, 14, 21*) were found too lengthy and cumbersome to be practical for determinations of water samples on a large scale, especially under field conditions.

Since the publication of the benzidine method (*12*) and its subsequent modification (*6, 9, 17*), many variations of the method have appeared, both colorimetric (*4, 8, 20, 22*) and titrimetric (*3, 5, 11, 13, 16, 18*). In the present application to total sulfur determination, recently published data for quantitative precipitation of benzidine sulfate have been incorporated. The volumes, concentrations, and time allowed for precipitation have been found necessary under the conditions of this method.

THE METHOD. Oxidation of the sulfur compounds with 30% hydrogen peroxide (Merck's Superoxol) was found most satisfactory, with no detectable interference with the benzidine method.

Into an evaporating dish containing a sample of water carrying between 3 and 20 mg. of sulfur, put 2 drops of 1% alcoholic phenolphthalein, add dilute hydrochloric acid if alkaline or sodium hydroxide if acid until the turning point of the indicator, then make the sample definitely alkaline with sodium hydroxide. Evaporate to about 5 ml., add 30% hydrogen peroxide, a drop at a time, and allow to simmer. After a few minutes, repeat addition of the peroxide until the solution becomes colorless or foaming ceases. Throughout this period maintain the volume of approximately 5 ml. by addition of distilled water.

Add 50 ml. of distilled water, heat the sample to 50° to 60° C., and add 10 ml. of benzidine reagent (11.2 grams of benzidine hydrochloride reagent grade, Eastman, and 11.6 ml. of concentrated hydrochloric acid, made up to 500 ml.). After a few minutes add 5 ml. of 99.5% acetone and let stand until cooled to room temperature. Filter through sulfate-free filter paper. Wash the evaporating dish twice with 5 ml. of 50% acetone and pour over the precipitate. After complete drainage, further wash the precipitate twice with 3 ml. of 99.5% acetone and let drain but do not allow to dry. Remove filter paper and wash the precipitate from the filter paper into the evaporating dish in which the precipitation took place. Heat to boiling and add 3 drops of 1% alcoholic phenolphthalein. With a microburet, titrate while hot with 0.01 *N* sodium hydroxide until near the end point, then boil again, and continue titration to first permanent pink color. Determine a blank on the reagents used.

DISCUSSION

Recoveries of known quantities of sulfur in various forms by this method are compared with unoxidized recoveries in Table I. The accuracy has been found to be ±0.2 mg. if the amount of sulfur present in the sample is between 3 and 20 mg.

Compounds such as *p*-aminodimethylaniline sulfate, potassium aluminum sulfate, and hydrazine sulfate have been tried with similar success. Mixtures of organic and inorganic sulfur compounds as found in paper-mill wastes lend themselves well to this method, providing all the material in suspension is first filtered out.

The sulfur content of pyrite waters from coal-mining operations can be determined as simple sulfate, with the reservation that iron in excess of 2 p.p.m. be removed from solution by precipitation. The iron, if not removed, oxidizes from ferrous to ferric and results in a fine dark precipitate, the presence of which alters the sulfate values.

Table I. Determination of Sulfur in Solutions Containing Known Amounts of Sulfur Compounds

Compound	Recovery before Oxidation with Superoxol			Recovery after Oxidation with Superoxol		
	Determined	Theory	Difference	Determined	Theory	Difference
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
Na_2SO_3	2.62	10.16	−7.54	9.96	10.16	−0.20
$NaHSO_3$	0.96	9.12	−8.16	9.24	9.12	+0.12
$Na_2S_2O_3.5H_2O$	0.21	10.32	−10.11	10.15	10.32	−0.17
$Na_2S_2O_4.2H_2O$	1.47	12.17	−10.70	12.29	12.17	+0.12
$Na_2S_2O_8$	10.01	10.76	−0.75	10.57	10.76	−0.19

ACKNOWLEDGMENT

The writer wishes to thank M. M. Ellis, in charge of the Water Quality Laboratories, Columbia, Mo., for his assistance and for the opportunity to write this paper.

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Separation of 2,4-Dinitrophenylhydrazones by Chromatographic Adsorption

JOHN D. ROBERTS[1] AND CHARLOTTE GREEN
Department of Chemistry, University of California
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IN DETERMINING the structure of a number of unsaturated compounds by ozonization, considerable difficulty has been experienced in separating small amounts of low molecular weight carbonyl compounds. The most satisfactory method of isolation is through the 2,4-dinitrophenylhydrazones, as these are readily formed and are insoluble in water, but in many cases separation of the derivatives by fractional crystallization is unsatisfactory.

Separations of a number of 2,4-dinitrophenylhydrazones by chromatographic adsorption have been reported by Strain (*4*), by Lucas, Prater and Morris (*2*), and by Buchman, Schlatter, and Reims (*1*), but only a few of the aliphatic derivatives have been investigated. In the present work, the adsorption of 2,4-dinitrophenylhydrazine and the 2,4-dinitrophenylhydrazones of acetaldehyde, propionaldehyde, acetone, and methyl ethyl ketone was studied on columns of silicic acid–Super Cel.

Procedure. The dinitrophenylhydrazones (*3*) were adsorbed from solutions in 1 to 2 benzene-ligroin (60° to 90°). The adsorbent was Merck's silicic acid (reagent grade) mixed with Super Cel in a 2 to 1 ratio by weight to increase the rate of flow of solvents through the column. The most satisfactory solvent for development of the chromatograms was ligroin (60° to 90°) containing 4% ether by volume. Mixtures of benzene and ligroin gave more diffuse bands. After the column was extruded and sectioned, the derivatives were eluted with ether and crystallized from ethanol.

In a typical experiment a mixture of about 5 mg. each of acetone and methyl ethyl ketone 2,4-dinitrophenylhydrazones was chromatographed on a 3.4 × 17 cm. column. After development the column consisted of the following bands: 4 cm. colorless, 4 cm. yellow, 2.5 cm. colorless, and 3.5 cm. yellow. The upper zone yielded the acetone derivative, m.p. 122–124.5° C. The lower band gave the methyl ethyl ketone derivative, m.p. 115° C.

The 2,4-dinitrophenylhydrazones were found to be adsorbed in the order: acetaldehyde, acetone, propionaldehyde, and methyl ethyl ketone. Each of these derivatives could be separated from mixtures with the others and 2,4-dinitrophenylhydrazine (strongly adsorbed) except for the hydrazone of acetone, which could not be separated from that of propionaldehyde.

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[1] Present address, Converse Memorial Laboratory, Harvard University, Cambridge 38, Mass.

Arrangement for Multiple pH Determinations

MAX D. REEVES, Northern Regional Research Laboratory, Peoria, Ill.

IN AN attempt to minimize the work required in making several hundred pH determinations a day, an arrangement has been worked out which facilitates this routine task to a marked degree.

The table in the assembly (Figure 1) is of desk height, in order to permit the operator to be seated while making the pH determinations. Figure 2 shows the construction and dimensions (in inches) of the table. For easier reading, the pH meter is set on a shelf at an angle of 15° to the table top.

The electrodes used were the No. 270 calomel electrode and No. 290 glass electrode as supplied with a Beckman Laboratory Model G pH meter. The leads on both the glass and calomel electrodes have been extended, so that they can be used at a distance from the meter case. The lead to the glass electrode must be shielded and the shield grounded to the case of the meter.

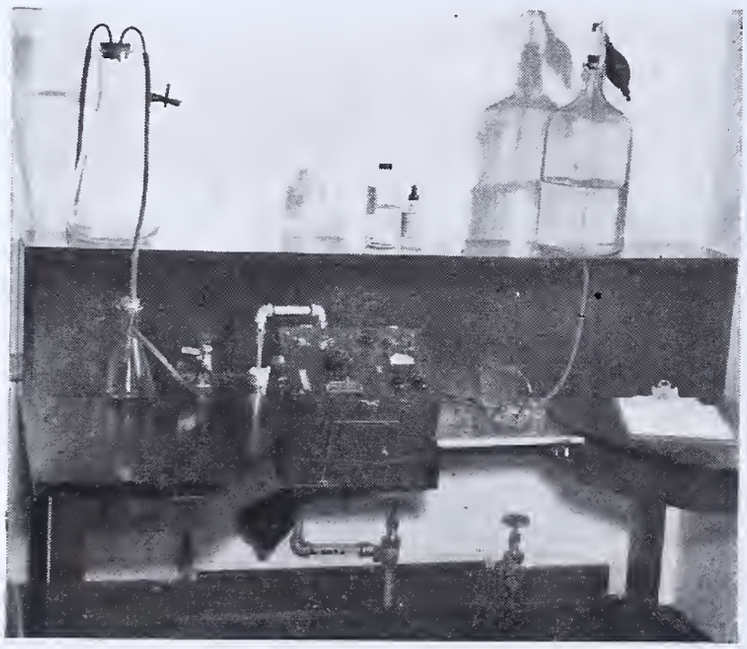

Figure 1. Assembly for pH Determinations

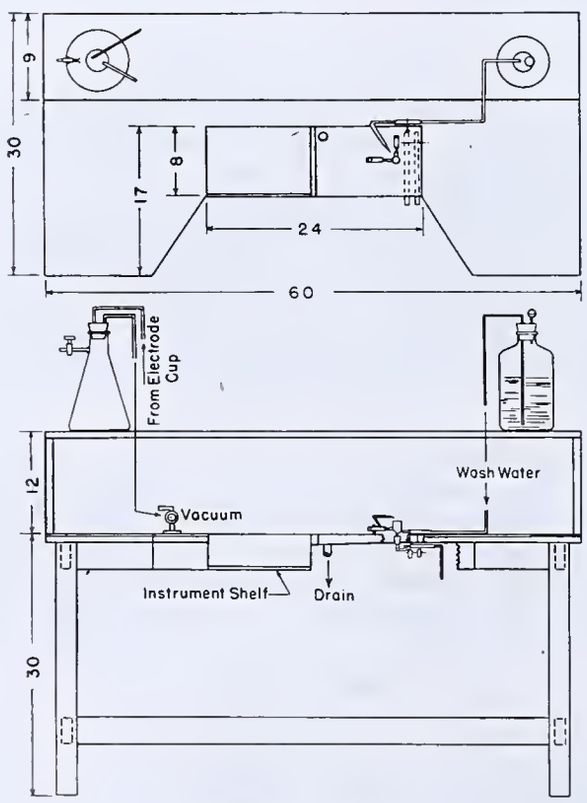

Figure 2. Table

The design of the electrode cup is shown in Figure 3. The purpose of the paraffin around the electrodes is to fill up the space between the electrodes and the cup wall, in order that less solution (only 2 ml.) will be required for the measurement and its subsequent removal facilitated. The hot paraffin is put in place with an eye dropper, the first arm being allowed to cool before the other is filled.

Figure 4 shows the details of the electrode cup assembly. Depressing the lever at the right causes the sample in the cup to be drawn by vacuum into the waste flask situated on the shelf at the back of the table (Figure 1). When the left lever is depressed, a jet of distilled water flows into the electrode cup. The washings are removed by depressing the right lever again. The copper pan is provided with a drain to permit easy washing and should be grounded electrically. When not in use the electrode cup is filled with water and covered with a small glass cap.

An indication of the accuracy of the equipment described is presented in Table I. Glycine–hydrochloric acid and boric acid–potassium chloride–sodium hydroxide buffer solutions were prepared (*1*). The pH of each of these solutions was first determined by the conventional procedure and then by use of the equipment described. These latter determinations were made in the order shown and as rapidly as possible. The number of rinses between determinations is indicated in the table.

Before each series of determinations the pH meter was standardized against 0.05 *M* potassium acid phthalate buffer solution (pH 4.00) and a standard buffer of pH 7.00 obtained from the National Technical Laboratories.

It is apparent from the values shown that the results obtained can be as accurate as the reading of the meter will allow. With but a single distilled water rinse between samples of widely differing pH, the largest difference noted was 0.05 pH, which is acceptable for most work.

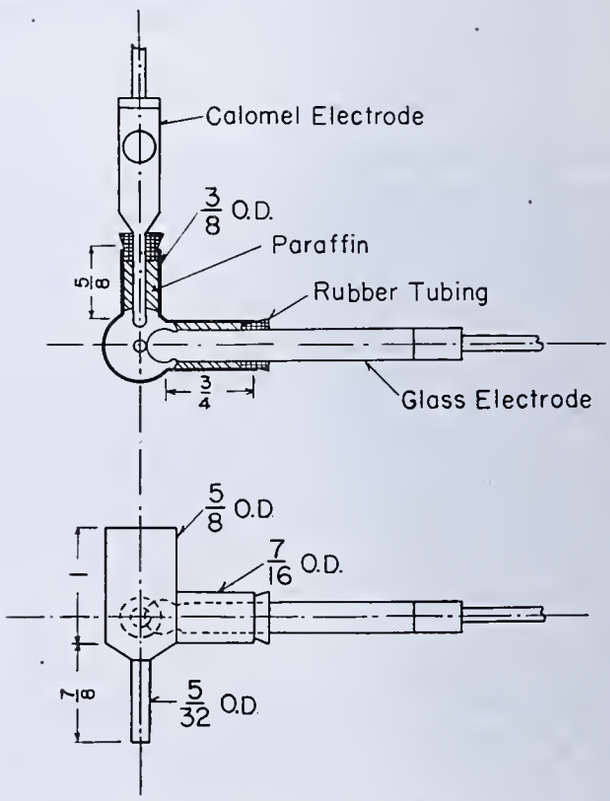

Figure 3. Electrode Cup Assembly

Dimensions in inches

Table I. pH at 25° C.

Buffer Solution No.	Given	Determined by Conventional Method	Determined by Present Method: One water rinse before each determination	Determined by Present Method: Three water rinses and three rinses with sample before each determination
1	10.0	9.97	9.90	9.95
2	3.99	3.97	3.98	3.95
3	8.8	8.77	8.74	8.75
4	1.93	1.97	1.92	1.90
5	7.8	7.75	7.72	7.73

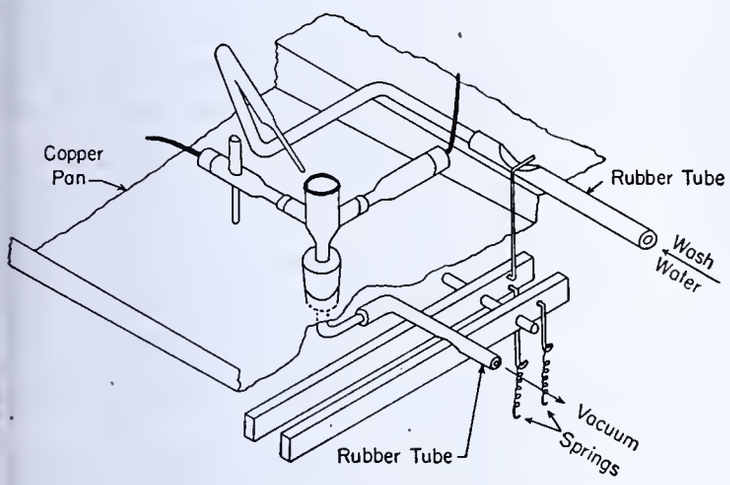

Figure 4. Electrode Cup Assembly

The electrodes were mounted in a horizontal position below the top of the electrode cup in order to obtain a clear working space above the electrode cup assembly. It was realized that contamination of the liquid junction solution might result with the calomel electrode mounted in this position. In order to establish accuracy of the instrument after use for 6 months, the system was checked against five buffer solutions prepared in accordance with the recommendations of the Bureau of Standards. Three of the solutions were prepared from chemicals supplied for this purpose by the Bureau of Standards. The other two solutions were prepared from A.C.S. reagent grade chemicals without further purification. The system was calibrated against buffer solution A and the results obtained on measurement of the pH of the other solutions are shown in Table II.

These data indicate that there has been little deterioration of the electrodes in the period of time they have been used. It is recommended that regular attention be given the saturated potassium chloride liquid junction solution in order to avoid errors due to dilution or contamination. To test the system for linearity, it may be calibrated against buffer solutions B and C, as recommended by Manov (*2*). The use of buffers of pH above 7.00 demands that precautions be taken to prevent absorption of atmospheric carbon dioxide. For normal use the system should be calibrated against a single buffer solution having a pH approximately that of the sample to be examined. Solution A or B will serve well for most purposes.

Table II. pH Measurements

Buffer Solution	Description of Solution	pH at 25° C. Given	pH at 25° C. Determined	Error
A	0.025 *M* potassium dihydrogen phosphate, disodium hydrogen phosphate, B.S. No. 186	6.86	...	...
B	0.05 *M* potassium acid phthalate, B.S. No. 84b	4.00	4.03	+0.03
C	0.01 *M* borax, B.S. No. 187	9.18	9.17	−0.01
D	0.05 *M* potassium acid phthalate, A.C.S. reagent grade	4.00	4.02	+0.02
E	0.01 *M* borax, A.C.S. reagent grade	9.18	9.18	0.00

The use of this equipment permits an unskilled operator to make from 100 to 150 pH determinations per hour. The electrode cup and electrodes may be rinsed well between determinations, and there is little danger of breaking them.

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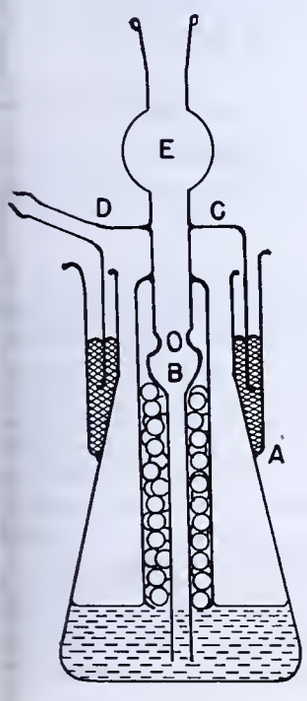
Modified Gas-Absorption Apparatus

ARCHIE N. BOLSTAD AND RALPH E. DUNBAR, North Dakota Agricultural College, Fargo, N. Dak.



RECENT modifications and improvements have been added to the gas-absorption apparatus recently described (*1*). Some difficulty has been encountered in adjusting the level of the absorption column to the changing level of the liquid. If the connection at the opening of the flask is sufficiently tight to prevent leakage of gas, it is almost impossible to make the necessary adjustments in level. This difficulty has been largely eliminated by adding the familiar liquid seal to the apparatus.

A small Pyrex beaker, with bottom removed, is sealed to the upper portion of a wide-necked Erlenmeyer flask as shown at *A*. This operation is not so difficult as it might appear, for the bottom of a small beaker can be readily removed by heating and blowing. The original pourout provides a convenient means for removing any sealing liquid used, provided the Erlenmeyer flask is first closed with a solid cork or stopper. The absorption portion of the apparatus, *B*, is identical with that previously described (*1*). A smaller beaker, *C*, is attached to the upper portion of the absorption tube, in an inverted position, as shown. A small inlet tube, *D*, is provided for introduction of the gas being employed. Mercury or any other heavy inert liquid may be employed for the seal. Obviously the liquid used must not react chemically with the gas employed. If a bulb, *E*, is constructed above the absorption tube as shown, it will largely eliminate the danger of overflow due to bumping or refluxing, and will also care for some increase in volume of the liquid.

With this modified gas-absorption apparatus the necessary adjustments can be made by raising or lowering the Erlenmeyer flask only. This is a decided advantage, since no other portion of the entire train of equipment is disturbed. The size of the equipment can be varied and proportioned to meet various needs.

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Electromagnetic Stirring Device

WILLIAM N. McINTOSH
Department of Physics, Howard University, Washington, D. C.

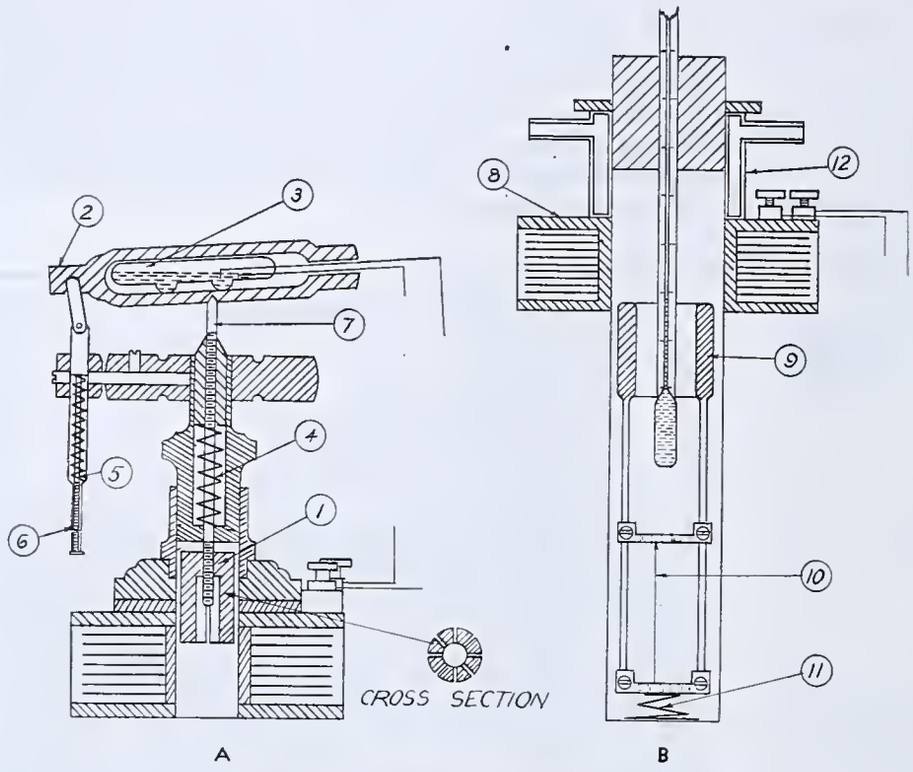

Figure 1. Diagram of Stirring Device

THE application of an electromagnetic stirring device is especially advantageous for agitating a liquid under pressure or in a vacuum.

Several articles describing such devices have been published most recently (*1–4*). The electromagnetic stirrer devised by the writer embodies simplicity of construction, reliability of operation, safety in use because of sealed contacts, and low operating temperature and cost. It can be used on 110-volt direct or alternating current, requiring 0.4 ampere and a 75-ohm line resistance on direct current and 0.7 ampere on alternating current with no line resistance.

The stirrer consists of two parts, *A* and *B*. The base of *A* is a solenoid directly connected to the 110-volt alternating current line or through the 75-ohm resistance to the 110-volt direct current line. The solenoid is made of a brass spool with 2333.4 feet of No. 24 enamel-covered copper wire (0.022-inch diameter) which operates a plunger made of solid soft iron (height 1.25 inches, diameter $^{15}/_{16}$ inch).

When the plunger, 1, is pulled down by the solenoid it lowers a lever, 2, consisting of a brass tube in which an evacuated mercury interrupter, 3 (available at laboratory supply houses as made by General Electric, Minneapolis-Honeywell Regulator Co., and American Instrument Co., Washington, D. C.), is situated, which breaks the current when the lever reaches its lowest point, thus allowing the lever and plunger to be pushed up by a coil spring, 4.

The tension of coil spring 5 at the end of the lever, which can be changed by manipulation of a screw, 6, regulates in a very simple and reliable way the time interval between the make-and-break of the current. The height of the pivot, 7, can also be regulated to give the mercury interrupter the proper inclination.

A regulates the speed of the make-and-break for an efficient stirring, whereas *B* is a stirring device activated by the intermittent current supplied by *A*. The solenoid, 8, acts on the soft iron cores of the stirrer, 9, inside the closed apparatus, inducing an up and down motion of the stirrer. The solenoid is made of short brass pipe 1.5 inches long, wall thickness 0.12 inch, brazed to a mild steel round plate 5 inches in diameter and 0.12 inch thick. It has 1400 feet of No. 24 enamel-covered copper wire.

The liquid is stirred by perforated plates, 10, attached to the stirrer, which is made (as is the whole apparatus) from nonmagnetic metal (copper or silver). This method of stirring is more efficient than rotating motion which has a tendency to move the entire liquid.

A spiral spring, 11, situated at the bottom of the apparatus intermittently touches the lower perforated plates, enhancing the regularity of the stirring.

A jacket, 12, through which liquids are circulated provides temperatures required by various experiments.

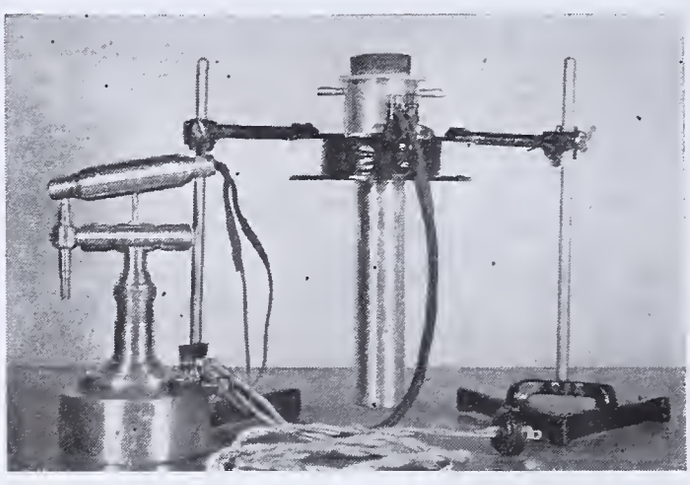

Figure 2. Stirrer

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BOOK REVIEW

Gas Analysis and Testing of Gaseous Materials. 1st edition *V. J. Altieri.* xi + 567 pages. American Gas Association, Inc., 420 Lexington Ave., New York, N. Y., 1945. Price, $5.00 to members; $7.50 to nonmembers.

The book has evolved from a revision of the widely used Gas Chemists Handbook of the American Gas Association and is the third of a series of volumes into which the former one-volume text has been expanded. It covers in a thorough manner that portion of the theory and practice of gas analysis customarily encountered by the chemists of gas companies (manufactured and natural gas). Gas analyses of types not often involved in the fuel gas industry are hardly more than mentioned. The book contains much in its 500 crowded pages that is related to but not usually considered a part of gas analysis—a concise outline of the kinetic theory, for example, and chapters on the measurement and flow of gases and on density.

The dominant impression the reader gets from the book is one of admiration for the thoroughness and rigidly logical operation of the author's mind and for his vast capacity for work. Everything is defined, classified, described, and pigeonholed with exactness, com-

pleteness, and finality. No chance is left for misunderstanding or error, and nothing essential to the discussions has been left out, no matter how obvious or well known it may seem to the reader. For example, a full page, containing both dimensional and differential equations, is devoted to "the concept of pressure". It is extremely doubtful that the discussion will be understood by any one who does not already have an entirely adequate concept of pressure. For this reason the book is tedious reading.

In his preface, the author shows that he set himself the task of writing a book that would meet the needs of every type of person concerned with gas analysis in the fuel-gas industries—to make "instantly available...detailed but concise directions, thoroughly indexed, for laboratory and plant technicians; theory, mathematical concepts, and literature references for chemists, physicists, and engineers; and matters of general interest for executives and persons who usually do not want highly specialized technical information". Perhaps the author's way is the only one in which this ambitious and very difficult objective can be attained.

Gas analysts are usually individualists, who tend to employ only apparatus and procedures to which they are accustomed and rarely publish data regarding the relative merits of competing analytical processes. The lack of such data, which is the principal defect of this very valuable text, is consequently not entirely the author's fault; but in the reviewer's opinion it should have been possible to give more information of the sort than has been included. The omission of some of the less useful equipment and procedures and the curtailment of some of the more generalized discussions would not be regretted.

E. R. Weaver

CORRESPONDENCE

Determination of *p,p'* Content of Technical DDT

Sir: In view of the publication of Cristol, Hayes, and Haller [Ind. Eng. Chem., Anal. Ed., 17, 470 (1945)] on the determination of *p,p'*-DDT in the technical material by crystallization involving the use of 75% ethanol, we wish to place on record the work done in England in the first half of 1943.

During our investigations on a suitable method for the manufacture of DDT, we succeeded in preparing material which could be readily isolated as a fine colorless powder, but which had a melting point range of 76° to 89° C., and although this was satisfactory for commercial purposes it was very desirable to work out a method which would give us the *p,p'* content, and therefore a measure of its purity. This was accomplished by a direct crystallization from 99.5% alcohol, under a set of standard conditions.

The method was communicated to the Ministry of Supply on Feb. 2, 1944, and in due course was incorporated in the British specification for technical DDT. Subsequently, work carried out by the Chief Chemical Inspectorate, Ministry of Supply, led to the use of 99.5% alcohol saturated with pure *p,p'*-DDT and also to an increase of the volume of alcohol employed, but fundamentally the method was unaltered.

Ten grams of the sample were treated with 30 ml. of 99.5% ethanol in a 100-ml. conical flask and brought to the boil to effect solution without loss of solvent. The flask was then set aside and cooled for 2 hours at room temperature (20° C.). The material was collected on a weighed No. 3 sintered-glass crucible, washed twice with two 5-ml. portions of 75% ethanol, and dried to constant weight at 80° C.

The following results were obtained:

Sample No.	*p,p'* Content, %	Melting Point, ° C.[a]
1	69.5	105–7
	69.5	105–7
	70.0	105–7
2	77.1	107–9
	77.8	107–9
	74.8	106–8
3	69.5	105–7
	69.9	105–7

[a] We had previously found that pure DDT had a melting point of 108.5–109.5° C. (corrected). Zeidler, *Ber.*, 7, 1191 (1874), recorded melting point as 103–105° C.

Pharmaceutical Laboratory
Geigy Co., Ltd.
Trafford Park
Manchester 17, England

I. E. Balaban
R. D. Calvert

Sir: The work described by us was conducted in the latter half of 1944 and makes use of 75% ethanol saturated with *p,p'*-DDT as recrystallization solvent. This gave more consistent results, in our hands, than that using stronger ethanol. We are happy, however, at this time to acknowledge the prior use by Balaban and Calvert of a recrystallization technique for the determination of the *p,p'* isomer content of technical DDT, earlier publication of which was barred by security restrictions.

Stanley J. Cristol
Robert A. Hayes
H. L. Haller

Bureau of Entomology and
Plant Quarantine
U. S. Department of Agriculture
Beltsville, Md.

Determination of Ethylene

Sir: We have tested the method of Francis and Lukasiewicz [Ind. Eng. Chem., Anal. Ed., 17, 703 (1945)] for the determination of ethylene and, while we found that the reagent they described absorbs olefins completely and rapidly, we also found that the reagent reacts at an appreciable rate with carbon monoxide to form carbon dioxide.

Samples of pure carbon monoxide and a mixture of 50% carbon monoxide and 50% nitrogen were tested with the mercuric sulfate solution in a pipet packed with vertical tubes, leaving the sample in the pipet 20 seconds between passes. After 20 passes had been made into the solution the sample was passed into 50% potassium hydroxide solution. The results of these tests are given here:

Passes	Pure CO *Ml.*	Pure CO *Ml.*	Mixture (50% N_2) *Ml.*
0	98.8	98.4	97.6
5	98.8	98.4	97.6
10	98.7	98.3	97.6
15	98.6	98.1	97.5
20	98.3	97.9	97.4
Passed into 50% KOH	93.1	92.3	95.4

The action of this reagent on carbon monoxide, forming carbon dioxide, may lead to erroneous results when an alkaline absorbent is used after the mercuric sulfate (such as alkaline pyrogallol solution for determining the oxygen content of a sample) or when the residual gas after absorption in mercuric sulfate solution is used for the determination of paraffin content by measurement of the carbon dioxide produced by combustion analysis. Errors of this type can, of course, be avoided by passing the gas into caustic solution after it is passed into the mercuric sulfate reagent and correcting any subsequent carbon monoxide determination by the amount of carbon dioxide removed.

F. R. Brooks
P. Benjamin
V. Zahn

Shell Development Co.,
Emeryville, Calif.

Sir: We appreciate the tests made by Brooks, Benjamin, and Zahn of our reagent for determining ethylene, indicating a possible error in its use, and also a means for avoiding the error. We believe their note will add to the value of the method.

A. W. Francis
S. J. Lukasiewicz

Socony-Vacuum Laboratories,
Paulsboro, N. J.

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Number consecutively and indicate reference in text by appropriate number in parentheses.

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Examples. (1) Comings, E. W., and Egley, R. S., IND. ENG. CHEM., **32**, 714–18 (1940).
(2) Li, K. C., and Wang, C. Y., "Tungsten", A.C.S. Monograph 94, p. 75, New York, Reinhold Publishing Corp., 1943.
(3) Smallwood, H. M. (to U. S. Rubber Co.), British Patent 533,669 (Feb. 19, 1941); Canadian Patent 403,992 (April 7, 1942).

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Manuscript Reviewing

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This is a promise to every contributor that his viewpoints—not the American Chemical Society's, the editor's, the reviewers', or the readers'—shall prevail in what is published under his authorship. By the same logic he, not they, must defend his position should his right to a place in the permanent literature ever be challenged.

With such apparent shift of credit or blame to contributors, the present rigorous review system of the Analytical Edition may seem superfluous. However, most authors, even though convinced of the value of their offerings, feel easier if they know others share their opinions. Likewise the editor, who has definite obligations to the American Chemical Society and to readers of the journal, wishes assurance from disinterested judges that a contribution merits space.

In the present system, hundreds of reviewers are called upon each year. No honorarium is provided and the privilege of prepublication reading often has dubious value, but the majority respond readily. They submit recommendations in a truly constructive spirit because of a sincere interest in maintaining and improving publication standards. They are not a fixed group maintained to review papers in each category of analytical chemistry. Instead every author, every analytical chemist, and certainly every specialist in fields touching analytical chemistry is a potential reviewer. Whether called upon frequently or infrequently, he is chosen only after several factors have been considered.

A manuscript to be reviewed is first checked on a number of points. Who beside the author is working or has worked in the field? What has been published recently? Has it been noted by the author? Has he overlooked any recent Analytical Edition articles? Are they perhaps unknown to him because they are "in press" or in an even earlier publication stage?

In answering such questions it is usually not difficult to find a number of possible reviewers. The problem then is to choose those most suitable. Advisory Board members at intervals receive a list of manuscript titles so that they can nominate appropriate critics. Authors themselves are encouraged to name those they regard as qualified. Particularly if an author feels his work has been misjudged by critics chosen originally, the editor welcomes both a rebuttal to the objectionable criticisms and suggestions as to reviewers considered competent. Every effort is made to consider all pertinent viewpoints and to reach a final decision the author can understand and endorse even if unfavorable. A disappointed author should not be a dissatisfied author.

Two reviewers are usually chosen for the initial review. One is ordinarily from the rather limited group of "authorities" in their special fields. The other may not be so widely known, but he, too, is selected for his special interest in the subject. Sometimes he can give more helpful advice, and comment in greater detail than the "authority", because of closer association with the problem discussed or even because he can give more time to the manuscript.

With each manuscript the critic receives a rating form on which he marks the contribution for originality, quality, appropriate publication place, and attention required before publication. In addition, detailed comments are requested for transmittal to the authors. Reviewers are urged to sign these, but only if they can write as freely over their signatures as otherwise.

This choice between being anonymous or named is a feature of the review system which many authors and some reviewers disparage. Any sincere critic, they reason, should not be afraid to identify himself. If unsigned comments are adverse, the apprehensive, sometimes skeptical, author often resents them and assumes such reviewers are incompetent. Such assumptions are generally erroneous. They indicate a lack of understanding of human nature and a lack of faith in the editor and his advisers.

The theory of frank and open criticism is admirable. However, situations where anonymity alone seems to guarantee honest opinions and true candor are easy to recognize. How many employers have not wished for its freedom when writing letters of recommendation? Many critics desire it when evaluating the contributions of a fellow scientist. Both the former student criticizing the work of his teacher and the nonexpert commenting on the work of an expert may experience reasonable hesitation about signing reviews even when they know their criticisms are valid. Free to say what they honestly think, they can concentrate on their reviewing without concern for what the authors' personal reactions may be.

The editor also knows that some critics cannot sign reviews because of company policies. He may question the reasonableness of such policies, but they are beyond his control. To allow them to prohibit cooperation from reviewers so restricted would surely deprive many authors of sources of superior criticism.

The anonymous review has been denounced because it fosters personal bias and indulges the acrimoniously inclined critic. These arguments can be refuted in most instances, for while the patience and good will of critics are sometimes tried by the shortcomings of authors, caustic reports are rarely submitted. Differences of opinion exist, but they are usually the result of differences in training, experience, and familiarity with Analytical Edition publication standards rather than personal bias or antagonism. Where personal prejudice exists, it can generally be detected and counteracted. It is not uncommon when reviewers submit conflicting reports, to seek the advice of a referee, to whom the manuscript and these reports are made available. One of the great advantages of the present system is the opportunity to obtain views from more than one person or group. This permits a far wider sampling of opinion than is possible with a small group of advisers, however expert each might be.

Following an initial review, comments received are passed on to the author. As a rule the editor makes no request except that reports be thoughtfully considered. The comments give the author an idea of what readers might think or say were his article published as submitted. Here is his opportunity to take care of their questions in advance. If errors have been discovered, they can be corrected. If the presentation was found obscure, it can be clarified. If a reviewer has misinterpreted data, steps can be taken to keep the reader from doing likewise.

Sometimes criticisms can be disposed of easily. Sometimes a considerable exchange of views is required before an author has justified his position or been convinced that his contribution is not suitable. Whatever the outcome, there is no attempt to coerce any author into altering his manuscript against his wishes. Any compromise he makes as a result of reviewer suggestions is his own acknowledgment of sound criticism. Any revised draft he submits is assumed to be one he, himself, approves, not one he has prepared to please the critics.

The primary purpose of the review system is maintenance of high publication standards for the Analytical Edition. It requires faith on the part of hundreds of authors and good will on the part of an even greater number of reviewers. Over many years it has proved its worth in countless instances. The editor and his assistants have faith that it will continue to do so.

Molecular Weight Distribution Data on High Polymers

Graphical Representation

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This article describes the preparation and use of a series of graph papers designed to convert cumulative molecular weight distribution data for high polymers into straight lines. These graph papers are based on a theoretical two-parameter molecular weight distribution equation derived by Schulz from statistical considerations of the mechanism of vinyl-type polymerizations. It was found that most of the published molecular weight distribution data on vinyl polymers, cellulose, and cellulose derivatives will give fairly linear plots on these graph papers. In no case, however, is a perfect fit obtained. This failure, in the case of vinyl polymers, is tentatively ascribed to the simultaneous operation of several kinetic mechanisms during polymerization, and/or to faulty fractionation techniques. Various mathematical criteria for judging molecular weight distribution data are included, as is also a brief reference to the general use of these graph papers on unsymmetrical frequency distributions.

EXPERIMENTAL studies on molecular weight distributions for high-polymer systems by the method of fractional solubility (solution or precipitation) yield a series of relatively homogeneous fractions (*29*). The mass of each fraction is added up in stepwise fashion to produce an S-shaped curve of cumulative percentage against polymerization degree of the individual fractions. This curve is then differentiated to obtain the molecular weight distribution curve.

Unless a large number of fractions have been obtained, some uncertainty will exist as to the exact shape of the S-shaped curve, and this uncertainty will be multiplied by the graphical differentiation step. The resulting error may not be serious for a general characterization of the distribution curve. However, it does become important when such data are used to draw conclusions about the kinetics of polymerization. For example, Flory (*10, 11, 12*), Schulz (*39, 41, 42*), Herington and Robertson (*18*), Ginell and Simha (*15*), Stockmayer (*52*), and Hulburt *et al.* (*20*) have derived theoretical molecular weight distribution curves based on specific polymerization mechanisms. Polycondensations, branching, chain transfer, and other types of chain termination are among the reactions treated by statistical and kinetic methods. The numerical parameters appropriate to such distribution functions can in principle be obtained from an experimental distribution curve. While there is probably no substitute for a large number of narrow fractions (*34*), the question arises as to what graphical aids can be employed in handling experimental data.

The similar problem which exists in other fields, as with distribution of particle sizes or with various types of statistical data, has been discussed by Austin (*3*) and more recently by Rissik (*37*), who point out the advantage of using probability graph paper which transforms the S-shaped cumulative percentage curve into a straight line. This graph paper has the units along the cumulative percentage axis spaced according to the integral of the Gaussian distribution function, while those along the other axis may have a linear or logarithmic interval. A linear plot on such paper immediately furnishes an idea about the type of distribution involved, the general goodness of the data, and several statistical quantities such as the mean size and the standard deviation.

These graph papers, which are commercially available, yield straight lines of cumulative percentage versus molecular weight data for some high-polymer systems, but fail in many cases. A new series of graph papers was therefore designed to cover the unsymmetrical distribution curves which in theory and practice are characteristic of most high polymers. This article is concerned with the design and properties of these graph papers and with some conclusions reached in using them.

DESIGN OF GRAPH PAPERS

The preparation of the desired coordinate scales proceeds most simply by a graphical transformation based on an S-shaped curve of the correct type. This original curve may be an experimental one which is judged sufficiently accurate to serve as a model. Irany (*22*) has followed this procedure in making viscosity-concentration plots of polymers, where the lack of an adequate theory demanded a purely empirical approach. However, the theory of molecular weight distribution functions presents a number of models from which to choose.

A rather general distribution function with two adjustable parameters has been proposed on several occasions for different purposes:

$$W(P)dP = \frac{(1-\alpha)^{b+2}}{(b+1)!} P^{b+1}\alpha^{P}\, dP \qquad (1)$$

where $W(P)$ is weight the fraction of material found in the interval dP, P is polymerization degree, and α and b are constants. Equation 1 describes a family of unsymmetrical distribution curves with a single maximum and with the long tail of the distribution occurring at high values of P. The shape of the curve and the location of the maximum depend on the numerical values of α and b. As α approaches unity, and as b increases, the maximum occurs at progressively larger values of P, while as b increases the curves become sharper and more symmetrical about the maximum. For most vinyl polymers, α lies between 0.99 and unity. b, which has positive integral values including 0, is primarily responsible for the shape of the curve if α is 0.99 or greater.

There are at least three possible interpretations of Equation 1.

Schulz (*41*) first derived this equation in connection with his chain coupling theory for vinyl polymers. In this case α is the probability for chain growth, while $b + 1 = K$ is the number of growing polymer chains which combine to form the final polymer unit. Thus, when chain termination occurs by mutual coupling, $b = 1$ or $K = 2$. When b is 0, Equation 1 reverts to Schulz's original derivation for vinyl compounds (*39*) and to Flory's (*10*) derivation for linear polycondensation resins.

Later, Mark (*28, 29*) suggested that Equation 1 might apply to branched polymers where each polymer molecule contained exactly b branches in addition to the so-called main polymer chain. This branched structure could presumably arise either through the coupling of $b + 1$ growing chains, or by the simultaneous growth of a single molecule in $b + 2$ directions.

Finally, a specific reaction mechanism or interpretation can be discarded and Equation 1 used simply to fit an experimental curve by adjusting parameters α and b. In practice it turns out that most of the existing molecular weight distribution data can be fairly well described by Equation 1. Hence, whatever the significance of the parameters, Equation 1 provides a suitable model.

Probability graph paper was therefore constructed on the basis f this equation by choosing appropriate values of α and b, calulating and plotting the distribution function of Equation 1, nd integrating graphically to obtain an S-shaped cumulative ercentage versus degree of polymerization curve. Regular inervals were selected along the cumulative percentage ordinate nd projected, via the curve, onto the other axis. These projected oints along the abscissas determined the intervals for the cumuative percentage axis of the probability paper. The other axis n the probability paper was then made linear in degree of polyerization.

Since both α and b may vary from one polymer type to another, s well as for different modes of preparation of the same polymer, is necessary to design a range of graph papers. Fortunately, he values of α are likely to fall within a fairly narrow range. Moreover, if α is greater than 0.99, the shape of the distribution urve is practically independent of α, but is determined mainly y the value of b. Second, as b increases, the distribution curve ecomes more symmetrical in shape, and its integral plots as a easonably straight line on Gaussian probability paper for b reater than 6 to 8.

It was found, for example, that the following eight graph paers covered a wide variety of polymers and experimental condiions:

1. $\alpha = 0.99$, $b = 0, 1, 2$
2. $\alpha = 0.995$, $b = 1, 2, 3$, and 4
3. Gaussian probability paper, Codex Book Company Catalog No. 3127

To give some idea of the sensitivity of these graph papers to ange in value of b, Figure 1 has been prepared. It represents eoretical cumulative percentage curves for $\alpha = 0.995$, $b = 1, 2, 3$, and 4, respectively, all plotted on a graph paper degned to give a straight line for $\alpha = 0.995$, $b = 1$. It is seen that b deviates by only one unit from the value on which the graph aper is based, the linearity is destroyed and the curve fails to pass through the origin. Depending on the accuracy of the experimental distribution data, use of this type of graph paper affords a convenient means of determining the parameter b. In addition to being straight, the line should pass through the origin with a definite slope which depends primarily on the value of b. This slope is such that the number and the weight average degree of polymerization should occur at definite cumulative percentage values which are shown below.

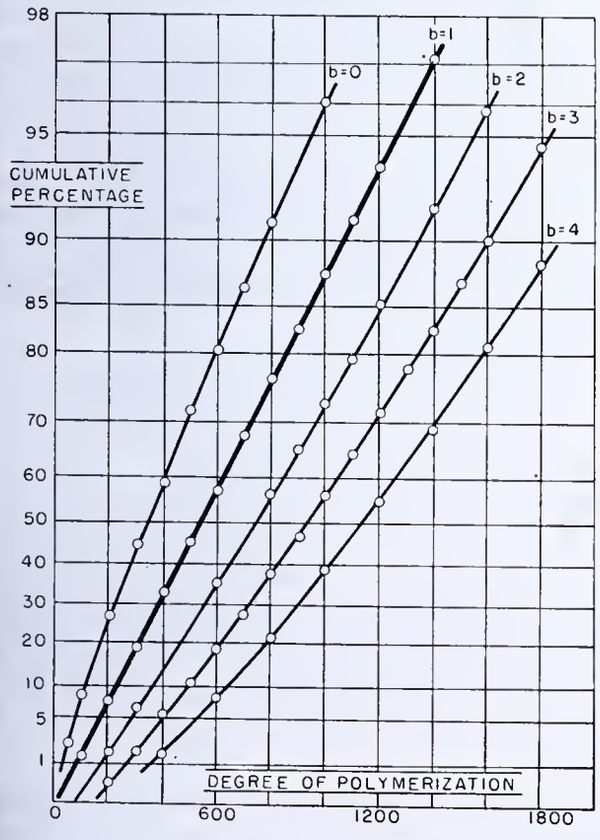

Figure 1. Calculated Cumulative Weight Percentage Curves

Based on Equation 1 for $\alpha = 0.995$, $b = 0, 1, 2, 3$, and 4, plotted on probability paper designed for $\alpha = 0.995$ and $b = 1$

Handling of Experimental Data. Following Schulz and Dinglinger (*43*) in calculating cumulative percentages, it is assumed that half the weight of each fraction is greater, half less, than the measured average molecular weight. Thus, the cumulative percentage corresponding to a given molecular weight or degree of polymerization is taken as the total weight per cent of all preceding fractions plus half the weight per cent of the fraction in question.

Once the data have been plotted on probability paper, a choice must be exercised as to the best straight line through the experimental points. Since the cumulative percentage scale is very expanded at each extremity, a large linear deviation in these regions parallel to the cumulative axis may not be serious. The linear polymerization degree scale affords a more convenient criterion for balancing deviations of the experimental points over all regions of the straight line.

The straight-line cumulative percentage curve can be differentiated numerically by employing the fact that the derivative at any point is proportional to the difference in scale reading on the cumulative percentage axis corresponding to a small, fixed interval along the degree of polymerization axis. It is more satisfactory, however, to replot values taken from the straight line back onto normal coordinate paper. This gives the S-shaped cumulative percentage curve which can be differentiated graphically, or with the aid of some mechanical device such as the tangent meter described by Richards and Roope (*36*).

There is always the danger, in work of this type, of trying to force a straight line onto data when such a procedure is not really justified by the data. The next section therefore considers certain criteria which will aid in making decisions on this point.

CALCULATION OF PARAMETERS α AND b

Before representing any molecular weight distribution data with these graph papers, it seems advisable to discuss some general features of the distribution function given by Equation 1, particularly in regard to methods of calculating parameters α and b from experimental data.

The value of b can be estimated from the type of probability paper which gives the best straight line for the experimental distribution data. There are cases when this method fails, particularly for high values of b, which require Gaussian distribution paper. In such cases, independent numerical checks on the value of b are needed. There are several general mathematical methods for finding α and b, such as the use of simultaneous equations, graphical approximations, etc. However, the unique properties of this distribution function suggest at least three special approaches to the problem.

A. Inflection Point Method. By setting the first derivative of Equation 1 equal to 0, the value of P corresponding to the maximum value of the ordinate is found to be

$$P_{max} = (b + 1)/(1 - \alpha) \qquad (2)$$

provided α is close to unity. (In general, the denominator of Equation 2 should be $-\ln_e \alpha$, which is valid for all values of α.) If b is 0, this reduces to the corresponding expression given by Flory (*10*) for condensation polymers. Next, the second deriva-

tive of Equation 1 is set equal to 0, and it is found that the inflection points come at

$$[(b + 1)/(1 - \alpha)] \pm [(b + 1)^{1/2}/(1 - \alpha)] \quad (3)$$

Thus the two inflection points are located symmetrically about P_{max}, and the separation, ΔP, between them, measured along the P axis is

$$\Delta P = 2(b + 1)^{1/2}/(1 - \alpha) \quad (4)$$

Elimination of α from Equations 2 and 4 gives an expression for b:

$$b = (2P_{max}/\Delta P)^2 - 1 \quad (5)$$

while

$$\alpha = 1 - (b + 1)/P_{max} \quad (6)$$

In theory, at least, one has merely to measure the separation between the inflection points and locate P_{max} in order to calculate both b and α. Actually it may be somewhat difficult to locate the inflection points exactly, and, since ΔP occurs as the square, the error in determining b will be correspondingly greater. Equation 4 is nonetheless a convenient criterion for inspecting distribution curves. If the curve is narrow (ΔP small) and if its maximum occurs at a high value of P, then b is large. If b is 0, there is but one inflection point on the descending side of the curve. Hence the presence or absence of an inflection point on the rising branch of the curve is immediately indicative of the general mathematical form of the distribution.

B. Areas under Distribution Curve. The total area under the weight distribution curve from $P = 0$ to ∞ is unity or 100%, but the areas from $P = 0$ to P_{max} in one case, and from $P = 0$ to $\bar{P}_w$ in the second case (where $\bar{P}_w$ is the weight-average polymerization degree), depend to a very good approximation only on the parameter b, if α is 0.99 or greater.

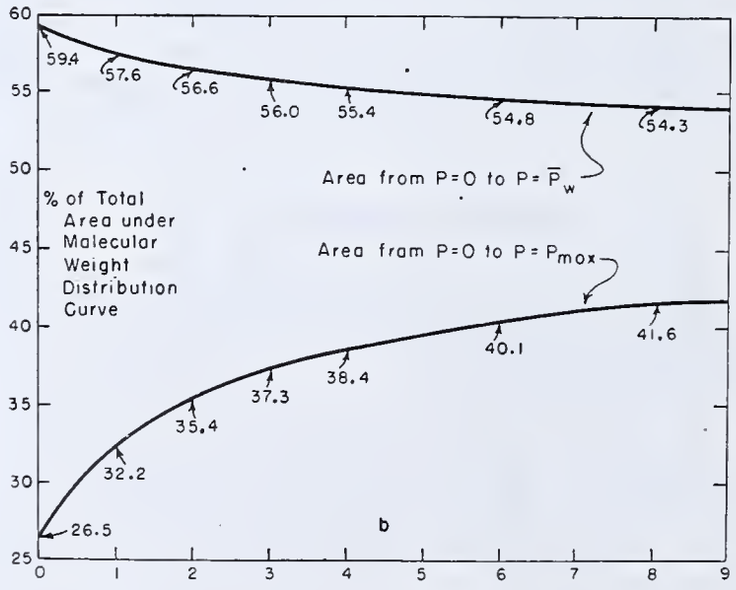

Figure 2. Areas under the Molecular Weight Distribution Curve

From $P = 0$ to P_{max} (lower curve) and from $P = 0$ to P_w (upper curve)

Specifically, by integrating Equation 1 and substituting the correct limits, it is found that:

$$\text{Area}]_0^{P_{max}} = 1 - e^{-(b+1)} \sum_0^{b+1} \frac{(b + 1)^q}{q!} \quad (7)$$

$$\text{Area}]_0^{\bar{P}_w} = 1 - e^{-(b+2)} \sum_0^{b+2} \frac{(b + 2)^q}{q!} \quad (8)$$

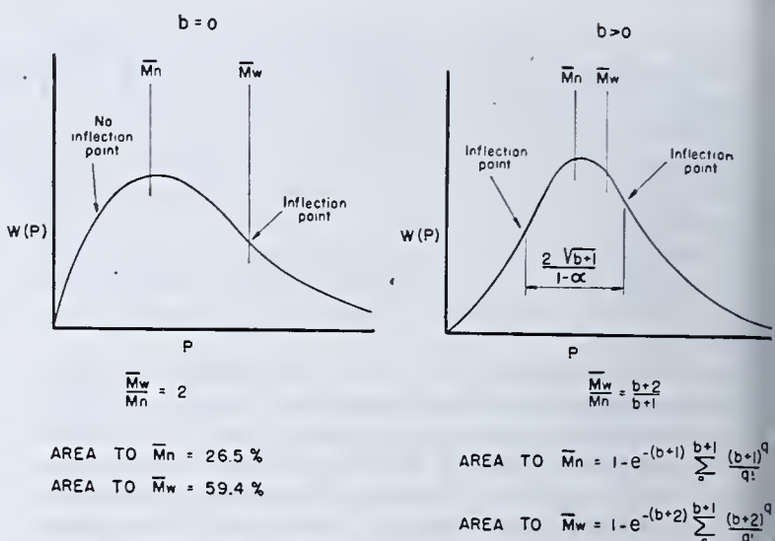

Figure 3. Characteristics of Molecular Weight Distribution Curve Described by Equation 1 for $b = 0$ and $b > 0$

These two functions have been plotted in Figure 2, from which values of b can be read if P_{max} or $\bar{P}_w$ is known. These are the relationships which fix the slope of the straight-line cumulative percentage curve mentioned above. Chowla and Auluck (*8*) have shown that the summation in Equation 7 approaches 0.5 exp $(b + 1)$ as b becomes very large. In other words, as b approaches infinity, exactly half of the area under the distribution curve is on either side of P_{max}.

C. Ratio of Weight-Average to Number-Average Molecular Weight. It is customary to calculate number, weight, and Z-average molecular weights for any molecular weight distribution function, according to the method of Lansing and Kraemer (*26*). For Equation 1, with α close to unity, there results:

$$\bar{M}_n = \bar{P}_n . M_0 = M_0 (b + 1)/(1 - \alpha) \quad (9)$$

$$\bar{M}_w = \bar{P}_w . M_0 = M_0 (b + 2)/(1 - \alpha) \quad (10)$$

$$\bar{M}_z = \bar{P}_z . M_0 = M_0 (b + 3)/(1 - \alpha) \quad (11)$$

where M_0 is the molecular weight of the monomer unit. $\bar{M}_n$ corresponds to the value of P_{max} given by Equation 2 while $\bar{M}_w$ is the average molecular weight determined by viscosity measurements if Staudinger's law holds. Otherwise $\bar{M}_w$ corresponds to the maximum in the Z-distribution curve $PW(P)$ which is the first moment of the weight distribution curve. $\bar{M}_w$ and $\bar{M}_z$ can be determined by ultracentrifuge studies. From Equations 9 and 10

$$\bar{M}_w/\bar{M}_n = \bar{P}_w/\bar{P}_n = (b + 2)/(b + 1) = R \quad (12)$$

or

$$b = (2 - R)/(R - 1) \quad (13)$$

Schulz (*41*) has used an expression of the form $R - 1$ to indicate the heterogeneity of a polymer. In his notation, $R - 1$ approaches 0—i.e., the sample is more homogeneous—first for narrow fractions, or second for polymers whose distribution curve follows Equation 1 with large values of b. Equations 12 and 13 likewise state that the polymer is more homogeneous—i.e., $\bar{P}_w/\bar{P}_n$ approaches unity—as b becomes large.

The main points in connection with these three methods of calculating α and b are summarized in Figure 3. It is characteristic of all methods that as b becomes large, the accuracy in determining its exact value diminishes. It is thus convenient to use these different methods for cross-checking. Moreover, the experi

mental weight distribution curve can be divided and then multiplied by the degree of polymerization to give the number distribution curve and the Z-distribution curve, respectively. The number distribution curve will have a maximum at $P = b/(1 - \alpha)$ if b is 1 or greater; otherwise at $P = 0$. The Z-distribution curve will have a maximum at $P = (b + 2)/(1 - \alpha) = \bar{P}_w$. Thus, the maxima in the number, weight, and Z-distribution curves are spaced along the polymerization degree axis in the ratio of $b:(b + 1):(b + 2)$.

The exact position of the maximum for the weight distribution curve is somewhat uncertain because it arises from the graphical differentiation of the cumulative percentage curve at its steepest part. However, the maxima for the number of Z-distribution curves derive from less steep parts of the cumulative weight percentage curve and should be less subject to error. Finally, the number distribution curve can be graphically integrated to give a cumulative number distribution curve. This should plot linearly on a probability graph paper having the same α but a b value reduced by unity compared to the graph paper used for the weight distribution curve. In similar fashion the Z-distribution curve can be integrated and should plot linearly on probability paper with b one unit greater than for the weight distribution curve.

While several checks on the values of b and α may appear unnecessary, actual experience in trying to apply these various criteria to existing distribution data has not been too successful. Several of the criteria may apply in a given case but complete agreement is generally lacking.

The foregoing treatment has assumed that true molecular weights are available for the fractions under study. However, most of the existing distribution data were based on Staudinger's linear relationship between specific viscosity, η_{sp}, molecular weight, M, and concentration, c:

$$\eta_{sp} = K_s M c \tag{14}$$

where K_s is the Staudinger constant. For many polymers this equation now appears to be seriously in error. An alternative viscosity law, originally proposed by Mark (*27*) and subsequently verified experimentally by Houwink (*19*), Flory (*13*), Mathes (*30*), and Alfrey, Bartovics, and Mark (*1, 5*) has the form:

$$[\eta]_{c=0} = KM^a \tag{14A}$$

$[\eta]_{c=0}$ is the intrinsic viscosity or limiting value of η_{sp}/c as the polymer concentration approaches 0, while K and a are constants. The value for a usually lies between 0.5 and 1.0.

Flory (*13*) has emphasized the fact that for polymers whose fractions obey Equation 14A, the molecular weight of the heterogeneous polymer determined by viscosity methods is not equivalent to the weight-average molecular weight, but gives rise to what he calls a viscosity-average molecular weight. A second consequence of the use of the Staudinger equation in cases where a is less than unity is that the distribution curve is compressed along the polymerization degree axis. It appears much narrower and covers a much smaller range of molecular weights than are actually present in the material. This apparent narrowing of the observed distribution curve forces one to choose a larger value of b than is required by the true molecular weight distribution.

There are several other devices which can be employed in checking on the accuracy of distribution curves. For instance, a comparison of the plots of η_{sp}/c versus c for fractions and for the heterogeneous polymer gives another check on the value of b in Equation 1 (*50*). Secondly, a detailed study of the number, weight, and Z-distribution curves allows one to calculate α and b of Equation 1 and a in Equation 14A. Both these techniques, which assume that the true weight distribution curve is described by Equation 1 are beyond the scope of this paper, and will be treated elsewhere.

APPLICATION OF PROBABILITY PAPER TO EXPERIMENTAL DATA

Mark has recently surveyed the literature on molecular weight distribution data, particularly in regard to cellulose and its derivatives, but also including vinyl compounds (*28*). Most of the data referred to by him, as well as some appearing since his article was published, have been plotted on these special probability papers. In general, a reasonably good fit could be obtained by the proper choice of graph paper, but in no case was there the complete accord between theory and experiment that might be desired.

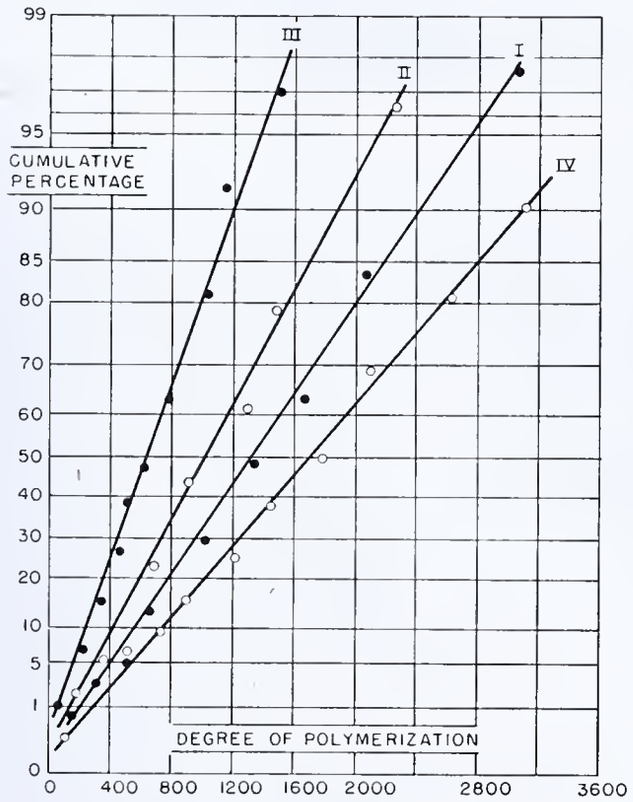

Figure 4. Cumulative Weight Percentage Curves

For four polystyrene samples of Schulz and Dinglinger listed in Table I. Probability paper has $\alpha = 0.995$, $b = 2$.

The most extensive and apparently most reliable set of results was that obtained by Schulz and Dinglinger (*43*) on four samples of polystyrene. Their data appear in Figure 4 using a graph paper with $\alpha = 0.995$, $b = 2$. Except for sample IV, all curves are reasonably satisfactory, although none of them passes through the origin. The fit for sample IV is considerably improved on a graph paper with $\alpha = 0.995$, $b = 4$. Sample II was fractionated twice, and both sets of results are in complete harmony, thus checking the consistency of their experimental technique.

Several fractions from each of these four polymers were measured for osmotic molecular weights, but the distribution curves are based on Staudinger's law. Recalculation of curves I and IV on the basis of Equation 14A (for which $a = 0.90$ was calculated from their viscosity–osmotic molecular weight data) made no perceptible improvement in the fit of the data. The straight lines still failed to pass through the origin, and still did not have the correct slope. A summary of the history of these four samples, together with calculations concerning them, is collected in Table I. It is evident that the values obtained for b show an extremely wide variation, but that the graph paper does a fair job of selecting a representative value.

Schulz and Dinglinger had concluded that these curves could

Table I. Summary of Calculations on Schulz and Dinglinger's (43) Molecular Weight Distribution Data on Polystyrene

Sample	I	II	III	IV
Polymerization temperature, ° C.	132	132	132	140
Solvent	Benzene	Benzene	Ethyl benzene	None
Styrene in solvent, %	20	20	20	100
Yield of polymer, %	11	67	69	60
$\frac{\eta_{sp}}{c}$ for polymer	7.2	5.9	3.4	9.2
$\sum \frac{\eta_{sp}}{c}$ for fractions	7.5	5.8	3.8	9.1
$\overline{P}_n$[a]	1020	815	520	1540
$\overline{P}_w$[b]	1500	1125	715	1880
b from $\overline{P}_w/\overline{P}_n$	1.15	1.60	1.65	3.55
b from probability paper	2.0	2.0	2.0	4.0
b from inflection points	2.76	2.27	3.0	3.85
b from area to Pmax	1.25	2.70	3.0	...
b from area to $\overline{P}_w$	0	0.70	0.70	1.50

[a] From peak in weight distribution curve.
[b] From peak in Z-distribution curve.

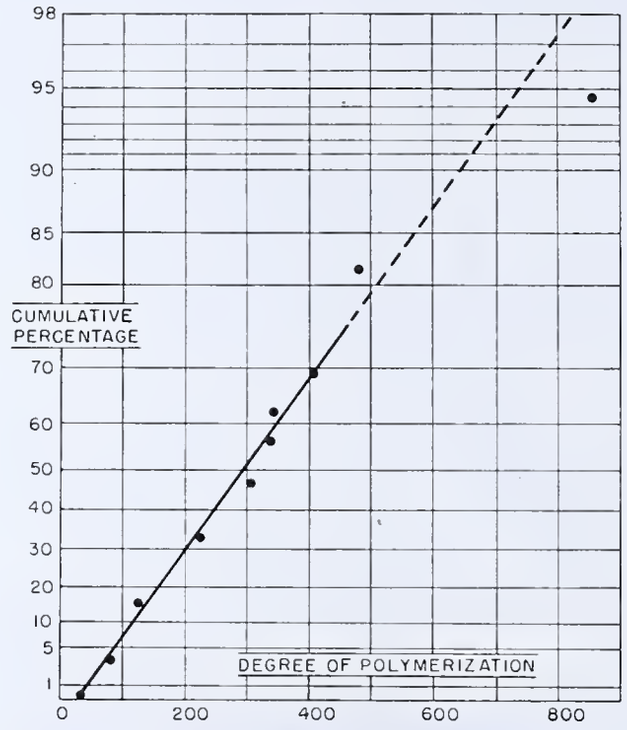

Figure 5. Cumulative Weight Percentage Curve for Polymethylmethacrylate

Data by Schulz and Dinglinger (44), graph paper of $\alpha = 0.995$, $b = 0$

be fitted with $b = 1$, and hence decided that the distribution arose from the mutual coupling of two growing chains. However, their experimental curves were sharper than their theoretical curves plotted on the same graphs (*43*) and calculated for $b = 1$. In fact, a b value of at least 2 is needed to account for the shape of their curves. In terms of branching, $b = 2$ means a main chain and two branches.

The same authors have also obtained a distribution curve on a sample of polymethyl methacrylate (*44*), polymerized as a 24% solution in toluene under air at 158° C. to a yield of 35.5%. Their data are plotted in Figure 5. With the exception of the highest molecular weight fraction, the results are reasonably well described on a graph paper for $\alpha = 0.995$, $b = 0$. Again the straight line fails to pass through the origin. The ratio between weight and number average molecular weight is approximately 2, which makes $b = 0$, but the areas under various portions of the distribution curve are not in correct proportion. Schulz and Dinglinger stated that their curve was satisfied by $\alpha = 0.99793$, $b = 2$, although their own efforts at curve fitting contradict this conclusion. For example, their plotted weight distribution curve lacks an inflection point on the rising branch, and this is consistent only with $b = 0$.

Figure 6 presents the data on 28 fractions of cellulose acetate obtained by Sookne *et al.* (*49*). The first fraction could not be redissolved to furnish a molecular weight. Figure 6, plotted on the

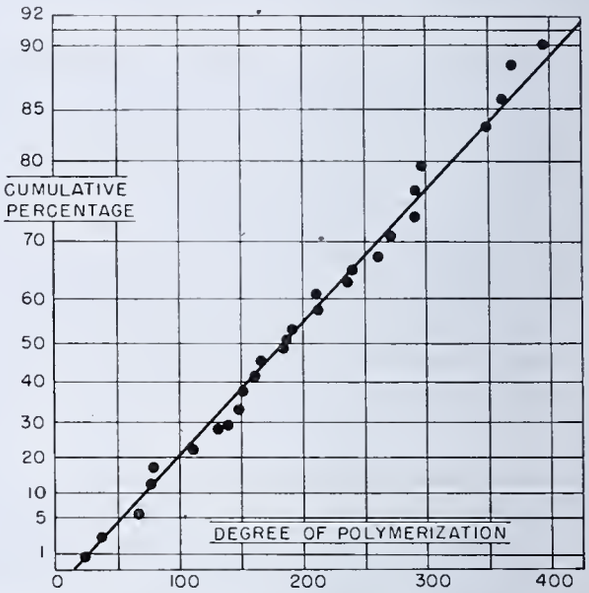

Figure 6. Cumulative Weight Percentage Curve for Cellulose Acetate (49)

Graph paper of $\alpha = 0.99$, $b = 0$

assumption that this was a true fraction, employs a graph paper with $\alpha = 0.99$, $b = 0$. If this first fraction is ignored, and the remaining fractions are spread over 100% total weight, a slightly better plot is obtained for $\alpha = 0.99$, $b = 2$. The behavior of cellulose itself is shown by Figure 7, where cumulative distribution curves for cotton cellulose and several chemically treated flax celluloses are plotted. These data, obtained by Straus and Levy (*53*) with cupriethylenediamine as solvent and sulfuric acid as precipitant, are best treated on Gaussian probability paper. The curves are narrow, thus corresponding to a high value for b.

There is no a priori reason for expecting cellulose and its derivatives to follow the same type of molecular weight distributions that obtain for vinyl polymers. It has been emphasized (*35*, *51*) that Spurlin's distribution curve on cellulose nitrate is considerably narrower than a representative distribution for polystyrene. Schulz's (*40*) work on nitrocellulose is further confirmation of this fact. To what extent this effect is real or merely a consequence of the application of Staudinger's law cannot be decided now. Gralen and Svedberg's (*16*) recent ultracentrifuge studies on the molecular weights of native cellulose indicate much higher values than had previously been estimated by viscosity on extrapolation of the Staudinger law. Moreover, the degradation of native cellulose incident to purifying it or preparing its derivatives will alter the original distribution considerably. The calculations of Montroll and Simha (*32*) predict that a very homogeneous starting material acquires on degradation various degrees and types of heterogeneity, not necessarily represented by Equation 1.

In addition to the specific examples cited above a more general survey of the results obtained in this study is tabulated in Table II. The number of fractions, the values of b from the graph paper and also from the ratio of weight to number average polymerization degree, and a qualitative evaluation (good, fair, bad) of the fit obtained on the graph paper are included. The last two columns list, respectively, the method of determining molecular weights (ultracentrifuge, osmotic pressure, or viscosity) and the literature source.

DISCUSSION OF RESULTS

In general, most of the avail-ble molecular weight distribu-on data can be accommodated ith some degree of satisfac-on on the various probability apers which have been pre-ared. However, in nearly very case the agreement be-veen theory and experiment ırns out to be largely illusory. more detailed study of the arious criteria listed earlier veals the following discrep-ıcies, even in cases where the ata lie on good straight lines.

1. The line does not pass ırough the origin.
2. The slope of the line is ot correct.
3. Some deviation from ıearity may occur at one or oth ends of the distribution ırve.
4. The areas under the distribution curve out to $\bar{P}_n$ and $\bar{P}_w$ e not consistent with the results given in Figure 2.
5. The ratio $\bar{P}_w/\bar{P}_n$ does not agree with the general shape of ıe distribution curve.

Some of these discrepancies, such as 4 and 5, are inherent in the ata and have nothing to do with the use of probability paper, or does it appear that a wider choice of graph papers would iminate the other discrepancies. These difficulties may arise ecause the distribution function of Equation 1 does not and ıould not apply, or because the fractionation data are at fault. everal possible errors would include use of Staudinger's equation ıstead of the more general Equation 14A, too few fractions, too igh concentrations of polymer during fractionation, degradation uring the experiment, improper choice of solvents, etc.

With the vinyl polymers it may well be that a series of istribution curves is superimposed. This could arise from the multaneous existence of two or more distinct polymerization ıechanisms, such as branching and chain transfer, each of hich mechanisms might give a different type of distribution urve. Herington and Robertson (*18*) have emphasized how ıe distribution curve will alter throughout the course of a olymerization for only one mechanism—namely, chain termina-on by the mutual coupling of two growing chains.

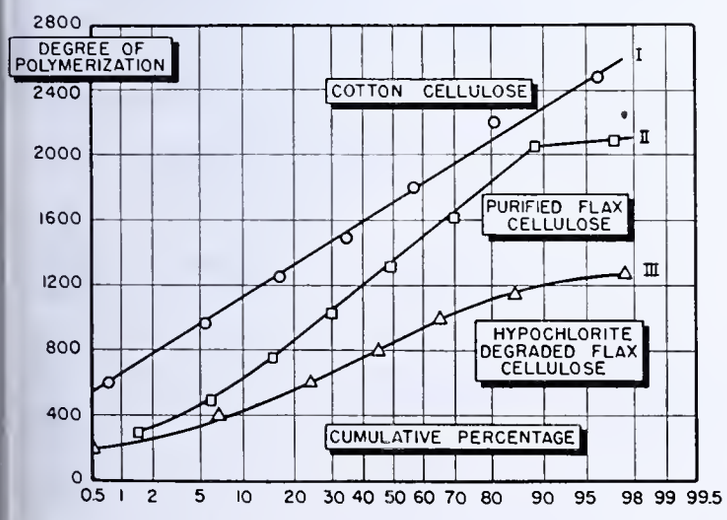

Figure 7. Cumulative Weight Percentage Curves for Several Cellulose Samples (53)

Gaussian probability paper

Table II. Summary of Fractionation Data on High Polymers

Polymer	No. of Fractions	Values of b^a From Graph Paper	Values of b^a From $\bar{P}_w/\bar{P}_n$	General Fit of Data[b]	Molecular Weight Method[c]	Reference
Polyisobutylene	8	0	0	F	V	(*39*)
Polymethyl methacrylate	10	0	0	F	V, O	(*44*)
Polystyrene						
Made at 90° C.	7	0	1	F	V	(*2*)
Made at 130° C.	6	∞	∞	F	V	(*2*)
Made at 132°–140° C.	8–10	2–4	1–4	F	V, O	Table I
Polyvinylchloride	5	∞	...	B	V	(*14*)
Polyvinylacetates	7	0–1	2–3	F	V	(*7*)
Polyvinylchloride-acetate	5	∞	...	F	V	(*9*)
Polymethacrylonitrile	6	...	...	B	O	(*24*)
Polyethyleneoxide	6	∞	∞	F	V	(*45*)
Buna S-benzene-soluble	..	0	...	B	U	(*23, 47*)
β-Amylose cornstarch	6	2	...	G	U	(*6*)
Gelatin	6	...	...	B	U	(*25*)
Nitrated Salep mannin	7	2	0	F	V	(*21*)
Methyl cellulose	11	2	1	G	U, V	(*48*)
Ethylcellulose	5	∞	...	F	V	(*33*)
Cellulose acetate	28	2	3	G	V, O	(*49*)
Cellulose nitrate	5	∞	2	F	V, O	(*40*)
Cellulose nitrate	66	2	2	G	V	(*35, 50*)
Nitrated cotton cellulose	6	∞	...	G	V	(*38*)
Cotton cellulose	7	∞	∞	G	V	(*53*)

[a] ∞ High value of b.
[b] B, bad; F, fair; G, good.
[c] V, viscosity; O, osmotic pressure; U, ultracentrifuge.

Several synthetic distribution curves were therefore calculated to determine their behavior. In one case it was assumed that a polymer consisted of two distinct species, each having its own distribution curve, such that there were three parts of polymer with $\alpha = 0.995$, $b = 2$, and one part of polymer with $\alpha = 0.995$, $b = 0$. The composite distribution curve was smooth and had but a single maxima. Its integral gave a perfect straight line (likely fortuitous) on graph paper of $\alpha = 0.995$, $b = 2$, but the line was rotated clockwise and did not pass through the origin. Moreover, calculation of the parameter b by the several methods previously enumerated gave values from 0 to ∞. It is thus conceivable that the observed behavior of some experimental molecular weight distribution curves may reflect the presence of different polymer species arising from several kinetic mechanisms, or from variable extents of branching.

A further source of disturbance would be a gradual increase or decline in the value of the average polymerization degree with the course of the polymerization. Thus, in Table I, polystyrene samples I and II carried to yields of 11 and 67%, respectively, differ in average polymerization degree by 20%. While there is no other apparent difference in the two distribution curves, it seems evident that each must represent the summation of many distribution curves. In this same series of polystyrene samples, the first three prepared in solvent showed on the average a lower b value than No. IV made from pure styrene. The greater prevalence of chain transfer in the presence of solvent (*31*) may well account for this difference in behavior.

In regard to branching, it seems fairly certain that if present it must affect the shape of the distribution curve. It is less certain that a fractional solubility technique will reveal the true picture of branching. Fractionation experiments isolate relatively narrow bands of molecular weights through the marked dependence of solubility on chain length (*46*). This may be rigorously selective for the members of a polymer homologous series but it may fail in the presence of branching, which would affect solubility independently of molecular weight. Thus, Baker, Fuller, and Heiss (*4*) have observed "abnormally high solubility" for slightly branched polymer molecules, as compared with linear molecules of the same chemical composition. These fractionation difficulties would be especially pronounced if one polymer contained several branched species or mixtures of branched and linear chains. Alfrey, Bartovics, and Mark (*1*) were recently able to separate a mixture of two polystyrene fractions each having the same osmotic molecular weight but coming from polymers prepared at different temperatures. While branching was not neces-

sarily involved in this experiment, some additional factor besides molecular weight affected solubility.

So long as all the branched species in a heterogeneous polymer are soluble, branching might be expected to sharpen a molecular weight distribution curve obtained by fractional precipitation. Thus, the largest molecules would on the average derive their size from numerous branches which would make such molecules more soluble than a linear molecule of the same molecular weight.

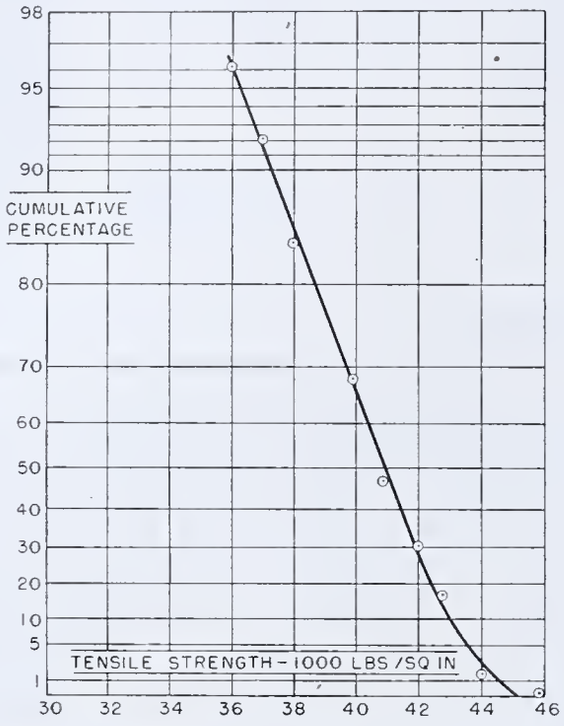

Figure 8. Distribution of Tensile Strength Values for 50 Samples of Saran Cordage

Ordinates represent percentage of samples which had not failed at indicated tensile load.

The smallest molecules would presumably be less branched and hence less soluble. The lowest and highest fractions would thus be shifted toward the center of the solubility distribution. Molecular weight determinations on such fractions might be in relative error because of the molecular complexity. It is hoped that the branching problem which is believed to exist in vinyl polymers can be treated more fully elsewhere. There is some possibility, at least with polystyrene, that branching may be connected with the activation step, but that linear molecules are also present through chain transfer.

The safest assumption concerning the distribution data treated in this report is that the probability graph papers have assisted in curve fitting, but that the data do not permit any decisive conclusions about polymerization mechanisms. (One might also say that a two-parameter equation such as Equation 1 is not sufficient to describe distributions resulting from the inhomogeneity of the kinetic process superimposed upon that inherent in the fractionation technique employed.) The existing data uniformly demonstrate the heterogeneous nature of the various high polymers studied, and do give an estimate of the approximate range of molecular weights present. It would seem advisable, however, to repeat all the experimental work under the guidance of current improvements in high-polymer theory and techniques.

APPLICATION OF PROBABILITY PAPER TO OTHER TYPES OF DATA

The special graph papers developed for molecular weight distributions can be expected to apply equally well to many other types of data which follow an unsymmetrical distribution law. One such example, given in Figure 8, applies to the range in tensile strength values obtained on breaking fifty samples of Saran cordage. Since the tail of this distribution curve runs off to the left, the cumulative percentage scale was plotted as samples that had not yet broken under a given tensile load. Harries, in his studies on glass, derived a probability law which suggested this application (*17*). The graph paper used was for $\alpha = 0.99$, $b = 0$, although these parameters do not have their usual significance in this application.

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Radioactive Studies

Analytical Procedure for Measurement of Long-Lived Radioactive Sulfur, S^{35}, with a Lauritzen Electroscope and Comparison of Electroscope with Special Geiger Counter[1]

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A general quantitative procedure for the measurement of S^{35} in tracer investigations is based on the oxidation of all sulfur-containing samples to the sulfate ion by the Carius procedure and subsequent precipitation as benzidine sulfate. This precipitate is collected in a specific manner and the activity therein determined with either a modified Lauritzen electroscope or a Geiger counter especially developed for soft beta-rays. A table and an equation are given for the self-absorption of the beta-particles in this precipitate. In certain respects, as compared to the Geiger counter, the modified electroscope is better suited to the routine measurement of low-energy beta-particles.

ALTHOUGH numerous investigators have used radioactive isotopes as tracers, no systematic effort has been made to develop simple quantitative procedures for determining these elements. Most investigations using radioactive tracers have been biological in nature and because of the inherent variations in biological work, analytical errors as large as 10 to 15% are not of too great import. There are four possible sources of error in the utilization of radioactive tracers in chemical and biological investigations: (1) the preparation of the sample for radioactive analysis, (2) the geometrical factors involved during detection of the disintegrations, (3) the statistical nature of the disintegration process, and (4) the detection apparatus used for determination of the radioactivity.

In numerous cases the errors resulting from the first two sources have been considerable; however, they are usually avoidable. The errors arising from the third and fourth sources have been subjected to mathematical analysis (*4*, *5*, *11*) and during routine radioactive analysis can be held to about 2%. Within readily recognizable limits, this error is essentially independent of the radioactivity present in the sample.

Certain radioactive elements are intrinsically more difficult to determine than others, owing to the nature and energy of their rays. Because of the low energy of the radiation, the following four radioactive elements are among the most difficult to determine quantitatively:

Element (*10*)	Maximum Energy of Emitted Particles *Mev.*	Half-Life *Days*
S^{35}	0.12 (β^-)	87
As	0.05 (I.T.e^-)	90
C^{14}	0.14 (β^-)	Approximately 1000 years
H^3	0.01 (β^-)	Approximately 30 years

Quantitative procedures (about 2% accuracy) have been worked out for the above low-energy-particle emitting isotopes—namely, the long-lived radioactive sulfur, arsenic, carbon, and tritium.

[1] First paper on subject "Radioactive Studies". Second will be found on page 354.

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This paper considers and evaluates in detail a routine quantitative analytical procedure that has been developed for 87-day sulfur. The radioactive sulfur used in the development of this scheme was obtained from the Radiation Laboratory, Berkeley, Calif.

The use of a radioactive isotope as a tracer depends upon the detection of a fractional number of disintegrations per unit time. In the case of S^{35} this means the accurate determination of a constant fraction of beta-particles of average energy of about 33,000 ev. This energy is so low that the betas are approximately one half absorbed by a 3 mg. per sq. cm. layer and about 95% absorbed by 10 mg. per sq. cm.

DETECTION APPARATUS

GEIGER COUNTER. The recent development of beta-ray Geiger counters (*2*) filled with helium at atmospheric pressure enables them to be constructed with extremely thin windows, which make them especially suitable for the measurement of soft radiation. This bell-type Geiger counter is shown diagrammatically in Figure 1.

The entire detection apparatus consisted of this Geiger counter, a counting rate meter (*6*, *9*), and an Esterline Angus recorder (Esterline Angus Co., Indianapolis, Ind.). Two minor improvements were made on the bell-type counter: The thickness of the mica window was reduced from the usual 5 to 10 mg. per sq. cm. to 2 mg. per sq. cm. This exceedingly fragile window (2.54-cm. diameter) was protected from the radioactive sample by means of a brass guard ring (1.91 cm., diameter). Before assembly, the copper cylinder was immersed in an ammonium polysulfide solution. The resulting black sulfide surface seemed to produce better plateau characteristics than the usual bright copper or copper oxide surfaces.

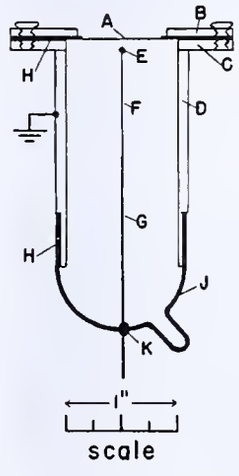

Figure 1. Bell-Type Mica Window Geiger Counter, Helium-Filled

A. Mica window (2 mg. per sq. cm.) coated with colloidal graphite
B. Brass guard ring
C. Copper support ring
D. Copper cylinder
E. 1/16 inch glass bead
F. 7-mil tungsten wire
G. 20-mil tungsten wire
H. Picein wax seal
J. Pyrex tube
K. Tungsten-to-glass seal

This counter had a threshold at about 1600 volts and a 180-volt plateau. It recorded about 60% of the S^{35} beta-particles impinging upon the mica window. When placed inside a 2.5-cm. (1 inch) thick lead box, the natural background (cosmic and local radiations) was 20 to 40 counts per minute.

MODIFIED LAURITZEN QUARTZ FIBER ELECTROSCOPE (*7*). An electroscope detects beta-particles through measurement of the ionization produced by the inelastic collisions of these particles with the air molecules inside the electroscope chamber. Since the path length of the low-energy S^{35} beta in air is not too long, a considerable fraction of the total ionization possible can be collected by the

electroscope unit. In fact, when samples are placed inside the electroscope chamber, the sensitivity to S^{35} of the Lauritzen electroscope is about the same as of the best designed bell-type Geiger counter.

A simple procedure for introducing the radioactive samples inside the electroscope chamber was worked out.

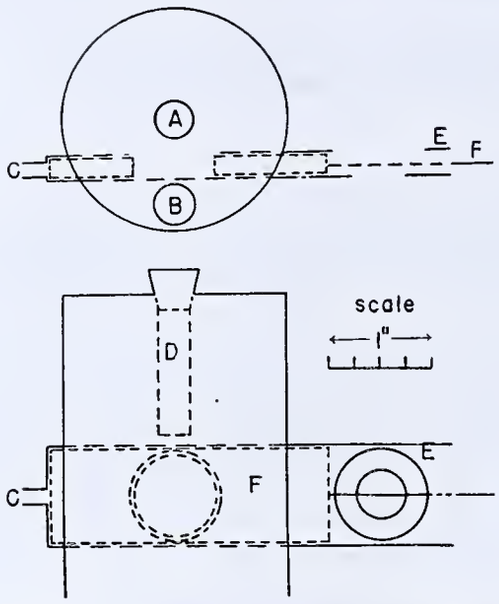

Figure 2. Modified Lauritzen Electroscope Case

A. Window
B. Opening for boat
C. To drying tube (magnesium perchlorate)
D. Boat for drying agent
E. Sleeve for sliding bar
F. Sliding bar for introducing brass disks

First the region of maximum sensitivity within the electroscope chamber was determined by placing a radioactive sample at various positions inside the chamber. Then a simple brass case was constructed which combined the usual cover of the ionization chamber with a sliding-bar device. This sliding-bar device enabled brass disks containing the radioactive samples to be introduced in the most sensitive location within the chamber in a reproducible manner. Brass was chosen as the construction material, since only about 6 alpha-particles are emitted per hour per sq. cm. of surface (*1*).

In order to increase the reproducibility of the electroscope, certain precautions were necessary:

1. A small boat filled with the drying agent, anhydrous magnesium perchlorate, was kept continuously inside the chamber; this resulted in much lower and steadier backgrounds. 2. The electroscope was kept in a balance case inside a balance room. The illuminating lamp burned continuously. These measures minimized temperature fluctuations to which electroscopes are somewhat sensitive. 3. When not in use the electroscope was kept continuously charged. Before use the electroscope fiber was discharged 2 or 3 times by means of a fairly strong sample. These measures ensured rapid attainment of electrostatic equilibrium. The power pack used to charge the electroscope consisted of a simple voltage-doubler circuit (25Z6 tube).

A diagrammatic representation of the modified case of the Lauritzen quartz fiber electroscope is given in Figure 2.

PREPARATION OF RADIOACTIVE SULFUR FOR MEASUREMENT

For accurate radioactive measurement, the low energy of the emitted beta-particles necessitates the isolation of a sulfur-containing compound in a pure state and the collection of this compound in a uniform and reproducible manner. The procedure described was developed with this in mind.

All the sulfur-containing samples are oxidized to the sulfate ion by the Carius method and the sulfur precipitated as benzidine sulfate; this quantitative procedure is described in detail by Niederl *et al.* (*8*). Instead of being evaporated to dryness under reduced pressure as suggested by these investigators, the Carius tube solution is transferred to a 30-cc. beaker and evaporated by means of either an infrared lamp or an inverted hot plate. Through experimentation it has been found that 6 to 7 mg. is a convenient amount of precipitate to handle. Thus enough inactive sulfur, usually as sulfate ion, was added to keep the benzidine sulfate weight above 6 mg.

A previously weighed filter paper circle, 2.22 cm. in diameter and prepared from Munktell's No. OK paper, is placed over a fritted-glass filtering disk of coarse porosity. The paper is wetted down with water, the water pump is turned on, and the glass cylinder is clamped in place with small springs; thus when assembled the filter apparatus resembles an Allihn filter tube (see Figure 3). This apparatus is somewhat similar to that described by Tarver and Schmidt (*12*). The benzidine sulfate is now rapidly washed into the glass cylinder. Final rinsings are made with 95% alcohol. The cylinder is removed and the water pump is turned off. The paper containing the precipitate is placed in a desiccator and reweighed, then permanently mounted by centering on a brass disk (19 mm. in diameter) and pressing on a brass ring which fits snugly over the disk (Figure 4). The activity of the precipitate is then determined by placing the assembled disk either inside the electroscope chamber by means of the sliding bar, or face down on the guard ring of the mica window of the bell counter.

Benzidine hydrochloride is superior to barium chloride as a precipitant, owing to the uniformity of the resulting matlike precipitate. The combination of Munktell's filter paper and the Corning fritted disk of C porosity results in a very uniformly distributed precipitate which adheres firmly to the filter paper. Numerous kinds of filter papers and fritted disks were tried before the above result was obtained. The filter paper with and without precipitate is reweighed after standing in a desiccator for one hour. Under these conditions the weights are reproducible to 0.05 mg. In order to prevent loss of weight due to handling, it is necessary that the filter paper have sharp edges; this is most readily accomplished by having the circles cut by a commercial stationery concern.

The importance of the above factors cannot be overemphasized. The energies of the emitted beta-rays are so low that a considerable fraction of these particles are absorbed in the benzidine sulfate. The uniformity of the precipitate reduces this self-absorption to a reproducible factor and the weighing of the precipitate enables this factor to be taken into account.

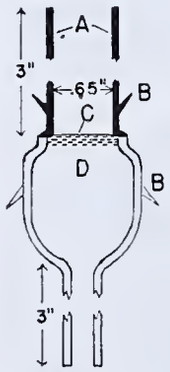

Figure 3. Benzidene Sulfate Precipitating Apparatus

A. Pyrex cylinder
B. Glass ears for springs
C. Munktell's No. OK filter paper circle
D. Fritted disk of coarse porosity; prepared from Corning GYCRY crucible

The procedure described above is well suited to routine use in both chemical and biological tracer work, since the Carius method enables a large number of samples to be oxidized simultaneously. By using a large-capacity electric furnace and a time clock, these oxidations can be done at night, thus enabling one person to make S^{35} analyses on about 20 to 40 samples per day.

Table I illustrates a typical set of S^{35} analyses for both a Lauritzen electroscope and a Geiger counter. Computations pertinent to columns 7, 8, and 9 are considered below.

RELATIVE ACCURACY AND SENSITIVITY OF RADIOACTIVE ANALYTICAL METHOD

The appearance of a beta-particle inside a Geiger counter produces a discrete impulse or "count" which is recorded by an auxiliary apparatus. Thus, the number of counts per minute minus the natural

Table I. Typical Set of S^{35} Analyses with Modified Lauritzen Electroscope and Bell-Type Mica Window Geiger Counter

Carius No.	Disk No.	Weight of Ppt., Mg.	Electroscope Reading Initial	Final	Time, t, Sec.	Total Activity $(d/t)_s \times 10^2$	Net Activity $[(d/t)_s - (d/t)_b] \times 10^2$	Weight Correction Factor (See Table III)	S, γ	S, γ per Unit Net Activity
			Electroscope 97, Expt. 19-P, 3/24/43							
	Std-1	7.00	45	55	173.1	5.74	5.68	1.000	3.200	0.563
			45	55	174.4					
	Std-2	7.00	45	55	292.4	3.41	3.35	1.000	1.900	0.567
			45	55	293.4					
12	7	8.70	45	55	312.1	3.21	3.15	0.895	1.99	...
			45	55	311.0					
14	9	6.07	45	55	100.5	9.91	9.85	1.065	5.27	...
			45	55	101.1					
17	11	11.25	45	50	1840	0.273	0.214	0.780	0.16	...
21	12	8.18	45	50	7100	0.070	0.011	0.925	0.007	...
	Background		45	50	8500	0.059	Nil	...	...	...
	Std-1	...	45	55	175.6	5.71	5.65	...	3.200	0.566
			45	55	174.6					
	Std-2	...	45	55	295.7	3.40	3.34	...	1.900	0.569
			45	55	292.1					
									Av.	0.566

Geiger Counter C and Counting Rate Meter, Expt. 19-P, 3/24/43

			Scale Reading		Total Activity, Counts per Min.	Net Activity $(c/m)_s - (c/m)_b$			
..	Std-1	...	3	69	3450	3420	1.000	3.200	1070
..	Std-2	...	3	41	2050	2020	1.000	1.900	1060
	Background		7	15	30	Nil	...	...	...
12	7	8.70	3	41	2050	2020	0.925	2.04	...
14	9	6.07	2	58	5800	5770	1.045	5.16	...
17	11	11.25	7	82	164	134	0.815	0.15	...
21	12	8.18	7	20	40	10	0.945	0.01	...
..	Std-1	...	3	68	3400	3370	...	3.200	1050
..	Std-2	...	3	42	2100	2070	...	1.900	1090
								Av.	1070

background is a relative measure of the amount of radioactivity present. This quantity will be denoted as

$$(c/m)_s - (c/m)_b \qquad (1)$$

where c denotes counts, m minutes, s sample, and b background. The appearance of the beta-particle in the neighborhood of the charged repelling post of a Lauritzen electroscope produces ionization which discharges the gold-plated quartz fiber at a faster rate than its natural (background) rate of discharge. Thus the reciprocal of the time for the fiber to move a certain number of scale divisions in the presence of a radioactive sample minus the reciprocal of the time for the fiber to move the same number of scale divisions in the absence of the sample (background) is a measure of the amount of radioactivity present. This quantity will be denoted as

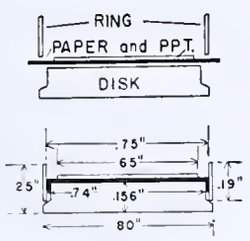

Figure 4. Brass Measuring Disks

***Above.* Unassembled**
***Below.* Assembled**

$$(d/t)_s - (d/t)_b \qquad (2)$$

where d is the distance traversed by the fiber in time t in seconds, s sample, and b background.

The relative sensitivity of the Geiger counter and electroscope may be defined for comparative purposes by the following equations:

$$\frac{(c/m)_s - (c/m)_b}{(\overline{c/m})_b} \qquad (3)$$

$$\frac{(d/t)_s - (d/t)_b}{(\overline{d/t})_b} \qquad (4)$$

where the bar in the denominator denotes average value. The sensitivity as defined by Equation 4 is essentially the same for all Lauritzen quartz fiber electroscopes. These electroscopes usually possess an absolute sensitivity such that 1 millicurie of radium at a distance of 1 meter produces a movement of 2 to 5 scale divisions per minute. Under these conditions all Lauritzen electroscopes discharge at a rate of about 60 times "natural" background. Thus the absolute sensitivity is of small import, since there is no appreciable loss in accuracy in allowing the fiber of the electroscope possessing a low absolute sensitivity to discharge 10 scale divisions instead of the 20 scale divisions one would use with electroscopes of greater absolute sensitivity.

The determination of experimental values for Equations 3 and 4 as a function of relative concentration of radioactive sulfur contained in a constant weight (7 mg.) of benzidine sulfate is a measure of the accuracy and relative sensitivity of the radioactive analytical procedure for S^{35} with both the electroscope and Geiger counter. The concentration of the radioactive sulfur was progressively diluted previous to precipitation by mixing known volumetric aliquots of both inert (carrier) sulfate ion and sulfate ion containing S^{35}.

Table II tabulates the results of duplicate determinations for both the electroscope and counter. The concentration of the weakest radioactive sample measured has been arbitrarily taken as unity. The data show that satisfactory radioactivity analyses can be made with samples containing activities 5 to 200 times the background. Since radioactive sulfur is readily obtainable with high specific activity, this is not a serious limitation of the procedure. The data indicate that the electroscope is somewhat more accurate than the counting apparatus used in this investigation. However, this difference would disappear on substitution of a well-designed scaling circuit for the counting rate meter. The relative sensitivities of the instruments are essentially identical; that of the electroscope could be increased somewhat by surrounding it with lead, 2.5 cm. thick.

The absolute sensitivity of both instruments is such that 10^{-4} microcurie of S^{35} will be of the order of the background in ac-

Table II. Radioactivity Measurements as a Function of S^{35} Content of Sulfate

(Dilution experiments. S^{35} concentration in least active sample is chosen as unity. Radioactivity measurements are expressed as ratio of net activity to average background.)

Modified Lauritzen Electroscope				Relative S^{35} Concentration C_i	Bell-Type Mica Window Geiger Counter			
Radioactive Measurements			Average deviation, %		Radioactive Measurements			Average deviation, %
Observed R_i	Av.	Expected[a] R_i			Observed R_i	Av.	Expected[a] R_i	
206	205	207	1.0	1000	253	254	254	0.0
204					255			
162	164	166	1.5	800	210	208	203	2.2
165					205			
103	103	104	1.0	500	125	126	127	0.8
103					127			
82.3	83.0	83.2	0.2	400	98	98.5	102	3.4
83.6					99			
41.8	42.0	41.6	0.8	200	50.2	50.6	50.8	0.4
42.1					51.0			
21.0	20.9	20.8	0.5	100	25.0	25.4	25.4	0.0
20.8					25.7			
17.2	16.9	16.6	1.5	80	21.3	20.8	20.3	2.2
16.5					20.2			
10.3	10.4	10.4	0.5	50	11.8	12.0	12.7	5.5
10.4					12.2			
8.23	8.15	8.32	2.0	40	11.0	10.7	10.2	4.4
8.07					10.3			
4.30	4.20	4.16	0.8	20	5.39	5.33	5.08	5.1
4.11					5.27			
2.07	2.15	2.07	3.6	10	2.39	2.48	2.54	2.4
2.22					2.56			
1.73	1.69	1.66	1.8	8	1.97	1.98	2.03	2.7
1.65					1.98			
1.12	1.09	1.04	4.3	5	1.35	1.31	1.27	2.8
1.05					1.26			
0.90	0.88	0.83	6.0	4	0.91	0.97	1.02	5.4
0.85					1.02			
0.41	0.44	0.42	4.8	2	0.46	0.50	0.51	2.0
0.47					0.54			
0.16	0.19	0.21	9.5	1	0.23	0.28	0.25	12
0.22					0.32			

[a] $R_i = \frac{C_i \Sigma (R_i/C_i) \log C_i}{\Sigma \log C_i}$. This equation expresses the fact that accuracy of measurement increases somewhat with concentration of radioactivity.

tivity. Thus 10^{-3} microgram of any sulfur compound can be readily determined if the specific activity is 1 millicurie of S^{35} per 10 mg. of compound.

CORRECTION FOR WEIGHT OF BENZIDINE SULFATE PRECIPITATE

Since the beta-particles emitted from S^{35} have a maximum energy of only 0.12 Mev., a considerable fraction of these particles are absorbed in the benzidine sulfate precipitate. Thus it is necessary to refer all radioactive measurements to a predetermined weight, which was arbitrarily chosen as 7.00 mg. or 3.25 mg. per sq. cm.

A correction chart was obtained by determining the radioactivity of constant concentrations of S^{35} as a function of the weight of the benzidine sulfate precipitate; the range was 1 to 25 mg. in 1-mg. intervals. All data are the averages of triplicate determinations (Table III). The values are not quite the same with the electroscope and the Geiger counter. Since the beta-particles are far from monochromatic and the Geiger counter counts each particle that enters the counter, while the electroscope measures the ionization resulting from a particle, the difference in the above results is to be expected. Further, the electroscope data are independent of the electroscope used, while the counter data depend upon the thickness of the mica window of the bell-type counter.

A consideration of the magnitude of these correction factors shows that the error due to self-absorption is not appreciable in radioactive analytical work, since the benzidine sulfate precipitate is weighable to better than 0.1 mg.

The self-absorption is expressed to an excellent approximation by the following equation (*3*):

$$I/I_0 = (1 - e^{-\alpha d})/2\alpha d \qquad (5)$$

where I_0 is a constant but may be considered the "true" total activity present in the benzidine sulfate precipitate, d is the "thickness" of the sample in milligrams per sq. cm., and α is an average exponential absorption coefficient of the following numerical value:

$$\alpha = 0.32 \text{ sq. cm. per mg.} \qquad (6)$$

for the electroscope data. This equation is derived in the following manner (Figure 5):

Let $2S$ be the "true" specific activity per unit weight (mg.) of precipitate. Assuming exponential absorption ($I = I_0 e^{-\alpha x}$), the measurable activity from the weight element, AdX, is

$$SAe^{-\alpha x}dx \qquad (7)$$

since $^1/_2$ of the disintegrations are directed downward.

Thus the total measurable activity is

$$I = SA\int_0^d e^{-\alpha x}dx = SA(1 - e^{-\alpha d})/\alpha \qquad (8)$$

Since "true" total activity is

$$I_0 = 2SAd \qquad (9)$$

we have by substituting (9) in (8),

$$I/I_0 = (1 - e^{-\alpha d})/2\alpha d \qquad (10)$$

All the approximations made in this derivation are trivial as compared to the assumption of exponential absorption of the beta-particles. This equation for the self-absorption of low-energy beta-particles which arise from radioactive disintegration seems to be experimentally valid to within 2 to 5%.

EVALUATION OF GEIGER COUNTER AND ELECTROSCOPE

The data given above show that accurate analytical results can be obtained with either the modified Lauritzen electroscope or

ell-type mica window counter. In fact, on the basis of accuacy and sensitivity they are essentially equivalent. Both instruents will readily detect 10^{-4} microcurie of S^{35}. In laboratories here there are a limited number of personnel trained in electronics, an electroscope is preferable in view of its ruggedness and implicity of design. It has been the authors' experience that uring routine use the entire counting apparatus is considerably ıore erratic in behavior than an electroscope. An electroscope ill give continuous reproducible results until the gold plating n the quartz fiber becomes broken; this phenomenon is readily ecognizable. Further, the cost of a complete counting apparaus is more than tenfold that of an electroscope. The average fe of an electroscope under the somewhat drastic conditions of lacing radioactive samples inside the ionization chamber is bout one year; thus it is advisable to own two electroscopes.

Two statements are sometimes made regarding the relative ıerits of the Geiger counter and the electroscope which require nsideration:

1. Since it is capable of detecting each individual disintegraon, a Geiger counter is intrinsically more sensitive than an lectroscope. This statement is certainly true of the theoretical ounter. Unfortunately it does not apply to the practical case f measuring low-energy beta-particles, since the routine placeıent of radioactive samples inside Geiger counters is not conucive to reproducible counting.

2. Since the accuracy of measuring activities of the order of ackground depends in part upon the reproducibility of this ackground, a Geiger counter is more accurate for these deterinations than an electroscope. This deduction is based essenally upon the fact that the time variation in the number of osmic and local rays entering a counter is less than the time ariation in the total ionization produced by these same rays as ıeasured by the electroscope (*4*, *5*, *11*). Actually the statistical ariations in the background of the electroscope are somewhat less than that of counters developed for the detection of soft beta-particles. This is due to the large difference in the "effective background detection area" of the instruments; the beta-ray counter enclosed in a lead box records about 30 background counts per minute, while the electroscope is measuring the ionization produced by at least 200 "background" rays per minute.

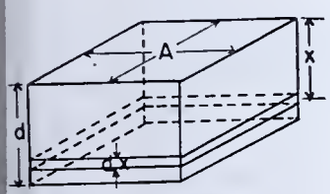

Figure 5

During two years, numerous background determinations have een made both with the electroscope and beta-ray Geiger counter. On a basis of 1-hour determinations, the daily variations ı background were with few exceptions never greater than 10% ith the beta-ray counter and 6% with the electroscope. Accurate determinations on samples containing activity of the order f the background can be made by measuring the sample, background, sample and background, respectively; this is how the ctivities in samples of concentration 1 to 8 in Table II were etermined.

Since the sensitivity with respect to background and the reproducibility of this background of the modified electroscope and ell-type mica window counter are essentially identical, the times equired to measure any radioactive sample to the desired accuacy with either instrument are equal. It is of interest to indicate he actual time of measurement. Samples with radioactivity of he order of the background necessitate 1 to 3 hours to determine o about 5%; however, with these samples this accuracy is usuılly not necessary and 15 minutes will suffice for most investigaions. The maximum accuracy for both counter and electroscope ill be with samples at least 10 times background. The time reuired to measure these samples is a matter of minutes. Because f the necessity of equilibrating the position of the quartz fiber, ccurate results with samples containing radioactivity in excess f 250 times background cannot be expected; depending on the lectroscope, an activity of this order will discharge the fiber at a rate of 10 to 20 small divisions per minute. The maximum counting rate for a Geiger counter depends on the over-all quenching circuit time constant. For the specific counter and auxiliary apparatus used in these investigations, this rate was 12,000 counts per minute (~400 times background).

Table III. Self-Absorption of S^{35} Beta-Particles

(Reciprocal correction factors for weight of benzidine sulfate precipitate for both an electroscope and Geiger counter. Net measured activity is to be divided by these factors. The factor for 7.00 mg. of precipitate is defined as unity.)

Electroscope Reciprocal Weight Correction Factor	Total Weight of Benzidine Sulfate Ppt. *Mg.*	Weight of Ppt. *Mg./sq. cm.*	Geiger Counter Reciprocal Weight Correction Factor
1.595	1	0.46	1.370
1.465	2	0.93	1.290
1.350	3	1.40	1.205
1.245	4	1.85	1.150
1.155	5	2.30	1.095
1.070	6	2.80	1.050
1.000	7	3.25	1.000
0.935	8	3.70	0.955
0.880	9	4.15	0.910
0.830	10	4.65	0.865
0.780	11	5.10	0.825
0.750	12	5.55	0.790
0.715	13	6.00	0.754
0.680	14	6.50	0.715
0.650	15	6.95	0.680
0.625	16	7.40	0.650
0.595	17	7.90	0.625
0.570	18	8.35	0.600
0.545	19	8.80	0.575
0.520	20	9.25	0.555
0.500	21	9.75	0.535
0.480	22	10.20	0.520
0.465	23	10.70	0.500
0.450	24	11.10	0.480
0.435	25	11.60	0.465

It will be shown in subsequent publications that the Lauritzen electroscope with certain modifications is well suited for the measurement of other radio elements emitting low-energy particles. Further, it is to be recommended for use with radioelements emitting high-energy particles, since it is usually not difficult to obtain these elements with sufficiently high specific activity at a reasonably low cost. If necessary, the electroscope chamber could be made gas-tight and filled with a high-density vapor such as sulfur dioxide or methyl iodide, thus increasing the ionization produced by the high-energy particles.

ACKNOWLEDGMENT

R. S. Halford of the Harvard Chemical Laboratories collaborated with the senior author in designing the modified case of the Lauritzen electroscope.

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Determination of Acetone

An Ultraviolet Spectrophotometric Method

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A method is described for the determination of acetone in mixtures with diisopropyl ether, isopropyl alcohol, and low-molecular weight mono-olefins. Essentially the procedure involves suitable dilution of the sample with 2,2,4-trimethylpentane, followed by measurement of the optical density of the diluted material at 280 millimicrons.

IN CONNECTION with the production of propylene by the catalytic dehydration of isopropyl alcohol, it was necessary to develop a rapid and accurate method for measuring acetone produced as a by-product of the main reaction. Low-molecular weight mono-olefins, diisopropyl ether, and unreacted isopropyl alcohol were also present in considerable quantities in the solutions to be investigated.

From the standpoint of both rapidity and accuracy, a photometric method, based on the absorption of ultraviolet light by acetone, appeared to be the most promising approach to the problem.

Hartley (*4*) has reported the complete absence of ultraviolet bands (above the Schumann region) for isopropyl alcohol. Bielecki and Henri (*1*) detected a very slight continuous absorption for *n*-propyl alcohol but, in the light of subsequent work by Massol and Faucon (*5*) who showed that the degree of absorption of a secondary alcohol was lower than that of the corresponding primary, it appeared that this absorption could be regarded as insignificant from an analytical viewpoint.

Carr and Walker (*2*) have thoroughly investigated the ultraviolet spectra of mono-olefins in the low-molecular weight range. In no case does the absorption become significant above 260 millimicrons, a wave length well below the acetone absorption maximum.

Despite a thorough search of the literature, no data on the absorption of diisopropyl ether were found. The absence of significant bands, however, has been proved experimentally.

The absorption of ultraviolet light by acetone has been the subject of much study and the authors' work has only verified previous reports (*6–9*).

EXPERIMENTAL

REAGENTS AND EQUIPMENT. Acetone, Commercial Solvents (C.P.).

Diisopropyl ether, Eastman Kodak No. 1193. This material was purified by successive fractionation and chemical treatment until only a very faint trace of acetone remained.

Isopropyl alcohol, Eastman Kodak No. 212, treated like the diisopropyl ether.

1-Octene, Connecticut Hard Rubber Company. Approximately 99% pure, the remainder consisting of close-boiling isomers.

2,2,4-Trimethylpentane (Iso-octane), Rohm and Haas material, carefully purified prior to use by means of silica gel (*3*).

All spectral measurements were made with a Beckman DUV spectrophotometer equipped with a hydrogen discharge source. The wave-length scale was calibrated with a mercury vapor lamp to within ±0.1 millimicron just prior to use. Matched quartz cuvettes (10 mm.) were employed to contain the sample and the 2,2,4-trimethylpentane reference solution.

SCANNING AND CALIBRATION. Figure 1 is a plot of experimentally obtained transmittancies against wave length for a solution of each component in the mixture. A band width of 0.5 millimicron was used throughout the entire wave-length range.

As can be seen, acetone showed a broad band centered at approximately 280 millimicrons. A calibration curve prepared at this wave length by plotting the so-called "optical densities" $\left(\log \frac{I_0}{I_x}\right)$ against the corresponding concentrations of acetone in 2,2,4-trimethylpentane showed no significant deviation from Beer's law.

CALCULATION AND INTERPRETATION

The molecular extinction coefficient, k, of each compound was calculated from the foregoing data, using the familiar modified form of the Beer-Lambert equation,

$$I_x = I_0 10^{-klc}$$

where l represents cell length (cm.), c the molar concentration and I_0 and I_x the incident and emergent light intensity, respectively. These data are shown in Table I.

SYNTHETIC SAMPLES

A series of synthetic samples containing varying amounts of the pure components was analyzed for acetone content. Each sample

Table I. Molecular Extinction Coefficients at 280 Millimicrons

Compound	Concentration *Vol. %*	Transmittancy %	k
Acetone	1	5.0	9.5
Olefin	10	87.8	0.0
Diisopropyl ether	25	93.8	0.0
Isopropyl alcohol	25	90.0	0.0

Table II. Synthetic Sample Data

Sample Composition				Results	
Alcohol %	Ether %	Olefin %	Acetone %	Acetone found %	Error %
95.0	...	...	5.0	5.38	+0.38
...	...	90.0	10.0	10.22	+0.22
...	85.0	...	15.0	14.86	−0.14
50.0	15.0	15.0	20.0	20.31	+0.31
10.0	50.0	15.0	25.0	25.40	+0.40
10.0	10.0	50.0	30.0	29.68	−0.32

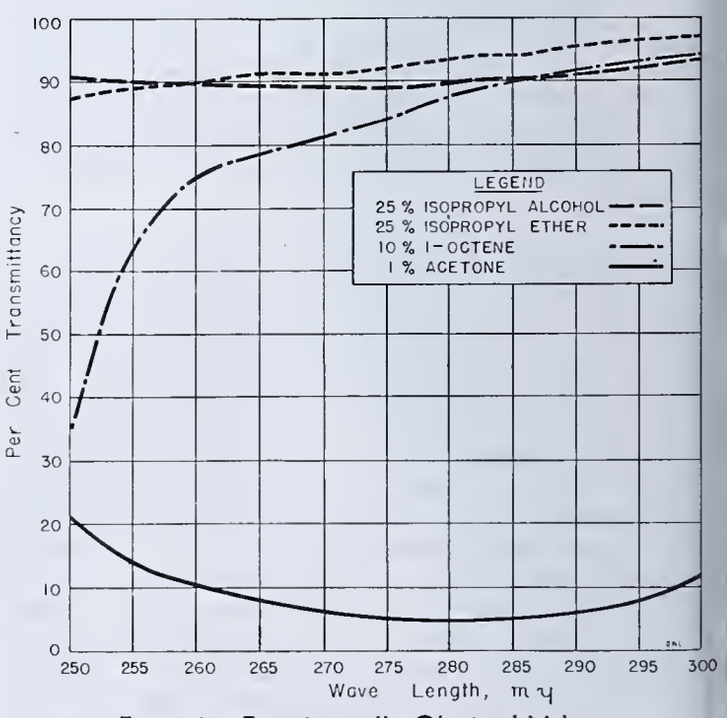

Figure 1. Experimentally Obtained Values

as accurately diluted with 2,2,4-trimethylpentane until the ptical density obtained fell within the range of the calibration urve. Reference to the curve and the application of the appro-riate dilution factor readily permitted calculation of the acetone oncentration in the original sample.

All values shown in Table II were calculated from the equation f the best-fitting calibration line. This equation was obtained y the method of least squares.

As can be seen from Figure 1, the rate of change of the extinc-on coefficient of acetone is small at its absorption maximum. his would suggest the ready application of filter photometers r transmittancy measurements despite the relatively broad ave bands usually associated with such instruments.

SUMMARY

Acetone may be determined in the presence of alcohols, ethers, nd mono-olefins of low molecular weight by measurement of the spectral transmittancy at 280 millimicrons. The data indicate that a low-cost filter photometer may be employed for obtaining satisfactory transmittancy values.

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Amperometric Titration of Chloride, Bromide, and Iodide Using the Rotating Platinum Electrode

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hloride, bromide, and iodide can be titrated rapidly in dilute solu-on with silver nitrate, using a rotating platinum electrode as the indi-ator electrode in amperometric titrations. Wide variations in the oncentration of acid or salts present do not affect the results. A ndency toward low results is observed with increasing dilution. hloride can be titrated in 10^{-4} *N* solution in 75% acetone, bromide 50% acetone, and iodide in water.

THE use of a rotating platinum electrode in amperometric titrations (*4*) of arsenite with bromate (*6*), silver with chlo-de (*6*), and mercaptans with silver (*3*) has been described. Hal-es can be titrated in a simple manner by measurement of the iffusion current of silver ions during the course of the titration. he end point is determined graphically from a plot of current gainst titration volume.

Salomon (*7*, *8*, *9*) made a study of "galvanometric titrations" sing two silver electrodes in dilute potassium chloride with an .m.f. of 0.1 volt applied across the electrode. When the otassium chloride was titrated with silver nitrate, practically o current flowed until an excess of silver had been added. The nd point was taken as the point where the sudden current in-rease occurred.

The dead-stop end point of Foulk and Bawden (*1*) is based pon the sudden polarization or depolarization of one or both lectrodes of an electrolytic cell in the presence of the first small xcess of reagent.

Both the galvanometric and dead-stop end points are very milar in principle to the amperometric end point. However, ne important new feature of the amperometric titration is that ne current is measured in general on a diffusion current region of current-voltage curve. On such a region, the current is inde-endent of the potential of the electrode because of an extreme ate of concentration polarization at the indicator electrode. ince the concentration of material undergoing electrode reaction maintained at a value practically equal to zero, the current is mited by the supply of fresh material to the electrode surface y diffusion. The rate of diffusion, and hence the current, is roportional to the concentration of diffusing substance in the ulk of the solution. By using a rotating electrode, the diffusion ayer thickness is decreased, thereby increasing the sensitivity nd the rate of attainment of a steady diffusion state. It is evident that a titration curve of diffusion current against volume of reagent is, in general, a straight line. (Strictly speaking, a correction for dilution is necessary to attain a linear relation between current and volume of reagent, but by working with a reagent which is tenfold more concentrated than the solution being titrated, the correction becomes negligibly small.)

In titrating halides with silver, the potential of the rotating electrode is made negative enough to plate out silver ions, leaving the layer of solution next to the electrode depleted of silver ions. The potential, however, must not be negative enough to give an appreciable current due to the reduction of dissolved oxygen. The range of permissible potential is strictly limited, as is evident from an examination of the current-voltage curves of silver-ion reduction and oxygen reduction using a silver-plated electrode (*6*). The potential of the saturated calomel electrode happens to lie in the permissible range, so that it is necessary only to short-circuit the rotating electrode through a suitable current measuring instrument to a saturated calomel electrode of relatively large area. Titrations in ammoniacal medium are carried out at a more negative potential, to deposit the complex diammine silver ions. Fortunately, the reduction curve of oxygen is shifted to more negative potentials in ammoniacal solution, and all that is necessary is to substitute for the saturated calomel electrode a reference electrode of more negative potential.

In the previous work on the amperometric titration of silver with chloride (*6*), a large irregularly fluctuating current was observed even in the presence of a large excess of chloride where the silver ion concentration should be very low. This current was attributed to a depolarization of the platinum cathode by colloidally dispersed silver chloride particles. It was found that the abnormal current could be decreased by flocculating the precipitate by the addition of electrolyte, and eliminated by the addition of gelatin. The gelatin causes the precipitate to become peptized by protective colloid action, but the gelatin-coated particles no longer are reducible at the cathode. The gelatin also changed the character of the plated silver from a coarsely crystalline to a finely divided, adhering deposit. With the coarsely deposited silver, a slowly drifting diffusion current was observed, while electrodes plated in the presence of gelatin gave constant diffusion currents for long periods of time. The drifting effect is unimportant in amperometric titrations if a freshly cleaned platinum electrode is used.

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Table I. Titration of 100 Ml. of Chloride

Normality of Cl^-	Normality of $AgNO_3$	Galvanometer Sensitivity	$AgNO_3$ Used Theory	$AgNO_3$ Used Experimental	Error
			Ml.	*Ml.*	%
Aqueous Solution, 0.8 *N* Nitric Acid					
0.100	0.500	1/50	20.00	20.00, 19.68 19.74, 19.87	0.0, −1.6 −1.3, −0.6
0.0100	0.100	1/50	10.00	9.99, 9.99 9.95, 9.99	−0.1, −0.1 −0.5, −0.1
0.0100	0.0500	1/20	20.00	19.82, 19.81 19.79, 19.86	−0.9, −1.0 −1.1, −0.7
0.0100	0.0500	1/50	20.00	19.99, 20.00 20.00, 19.99 19.97	−0.1, 0.0 0.0, −0.1 −0.2
0.0050	0.0500	1/50	10.00	10.01, 10.02 9.99, 9.99	+0.1, +0.2 −0.1, −0.1
0.0020	0.0100	1/50	20.00	20.00, 19.68 19.74, 19.87	0.0, −1.6 −1.3, −0.6
0.0020	0.0100	1/20	20.00	19.45, 19.46	−2.8, −2.7
0.0010	0.0100	1/20	10.00	9.36, 9.16 9.41, 9.10	−6.4, −8.4 −5.9, −9.0
0.0005	0.0100	1/20	5.00	4.25, 4.29 4.19, 4.20	−15.0, −14.2 −16.2, −16.0
50% Acetone, 0.8 *N* Nitric Acid					
0.1000	0.500	1/50	20.00	20.00, 19.97 19.98	0.0, −0.2 −0.1
		1/20	20.00	19.95	−0.2
0.0100	0.100	1/20	10.00	9.94, 9.94 9.93, 9.95	−0.6, −0.6 −0.7, −0.5
0.0100	0.0500	1/50 1/20	20.00 20.00	19.90, 19.89 19.95, 19.96 19.94, 19.96	−0.5, −0.6 −0.2, −0.2 −0.3, −0.2
0.0050	0.0100	1/50 1/20	10.00 10.00	9.96 9.95, 9.95 9.94	−0.4 −0.5, −0.5 −0.4
0.0020	0.0100	1/50	20.00	19.81, 19.83 19.82, 19.83	−1.0, −0.8 −0.9, −0.8
0.0010	0.0100	1/20	10.00	9.71, 9.71 9.73, 9.73	−2.9, −2.9 −2.7, −2.7
0.00050	0.0100	1/20	5.00	4.84, 4.83 4.83, 4.80	−3.2, −3.4 −3.4, −4.0
0.00020	0.0010	1/20 1/10	20.00 20.00	19.20, 18.70 18.87, 18.83	−4.0, −6.5 −5.7, −5.9
0.0001	0.0010	1/10	10.00	9.33, 9.05 9.08, 9.05	−6.7, −9.5 −9.2, −9.5
0.00020[a]	0.0010	1/10	20.00	19.45, 19.47	−2.8, −2.7
0.00010[a]	0.0010	1/10	10.00	9.75, 9.95	−2.5, −0.5

[a] In 75% acetone.

EXPERIMENTAL

Several types of rotating microelectrodes have been used, but the most satisfactory one in the authors' experience was made from a cone-drive laboratory stirring motor essentially as previously described (*6*). A sturdy piece of platinum wire (No. 18) was sealed into 6-mm. soft-glass tubing to form an electrode 5 to 10 mm. long. The tubing was bent at right angles about 2 cm. from the end. A drop of mercury inside the tube served to make electrical contact to a copper wire inside the glass tube, and connected at its other end to a brass sleeve inside the chuck provided with the motor. The electrical contact is very important and sliding or bearing contacts should be avoided. By inverting the shaft of the Sargent cone-drive stirrer, a small cup is formed at the top end of the shaft. A drop of mercury in the cup makes direct metallic contact between the rotating shaft and the frame of the motor which is provided with binding posts.

The sensitivity of the current-measuring device is of some importance, especially in chloride titrations. Owing to the solubility of the precipitate, an appreciable current passes through the cell at the end point and if a very sensitive galvanometer is used without shunting, a tendency toward low results is observed. A very convenient instrument for current measurements is the Fisher Electropode, which was designed for use with the dropping mercury electrode. It contains a multiple mirror lamp and scale galvanometer, provided with an Ayrton shunt which permits choice of a wide range of sensitivities. A microammeter of 15- or 30-microampere range, or a small enclosed lamp and scale type galvanometer, provided with a variable shunt, has been found entirely satisfactory. In general, the lowest sensitivity consistent with accurate readings has given the best results.

Care should be taken to arrange a reference electrode of large area (to prevent polarization by the passage of current) and salt bridges of low resistance. In the present work the electrode and salt bridge were of the type described by Hume and Harris (*2*). The agar plugs were replaced by plugs of sintered glass, prepared from powdered Pyrex (*5*). For titrations in ammoniacal medium, the mercury–mercuric iodide–potassium iodide cell, described by Kolthoff and Harris (*3*), of potential −0.23 volt *vs.* saturated calomel electrode, was used. In typical titrations, the entire cell had a resistance of the order of 1500 to 2000 ohms.

TITRATION OF CHLORIDE

Standard solutions of sodium chloride and silver nitrate were prepared accurately, using reagent quality salts which had been powdered and dried at 110° for 2 hours. Curves for the titration of 0.1 *N* and 0.001 *N* chloride are shown in Figures 1 and 2.

In a series of titrations of 0.01 *N* chloride, the concentration of gelatin was varied between 0.01 and 0.4%, nitric acid was added in concentrations between 0.08 *N* and 3.2 *N*, and sulfuric acid was added in concentrations between 0.08 *N* and 9.2 *N*. The maximum variation in results observed was 0.5%. Titrations of 0.002 *N* chloride in the presence of 0.2 *M* barium nitrate or 2 *M* potassium nitrate did not show interference by the added salts in the presence of 0.1% gelatin.

Table I shows data obtained for various concentrations of chloride in aqueous solution and in 50% acetone. In all these titrations, 100 ml. of chloride solution, 0.8 *N* in nitric acid, and 0.1% gelatin were used. The galvanometer sensitivity indicates the fraction of the full sensitivity (0.01 microampere per mm.) used in each titration.

According to Table I, satisfactory results can be obtained down to 0.002 *N* chloride in aqueous medium, but low results were found for lower concentrations (15% low at 0.0005 *N*). In general, the lowest galvanometer sensitivity consistent with accurate readings gave the best results, since the observations were taken relatively far from the end point in titration of dilute solutions.

Using 50% acetone as the titrating medium the solubility of silver chloride is reduced to such a point that 10^{-4} *N* chloride solution can be titrated with a 10% error. In 75% acetone, the error with 10^{-4} *N* chloride is reduced to 3%. However, the diluting effect of the additional acetone must be considered in making a comparison, since the original sample would be diluted fourfold using 75% acetone and only twofold using 50% acetone.

In general, the use of 50% acetone as a titrating medium is recommended for chloride concentrations below about 0.005 *N*.

TITRATION OF BROMIDE

The precision and accuracy of bromide titrations were checked under conditions very similar to those found suitable for chloride. Gelatin was added in the present experiments, although later

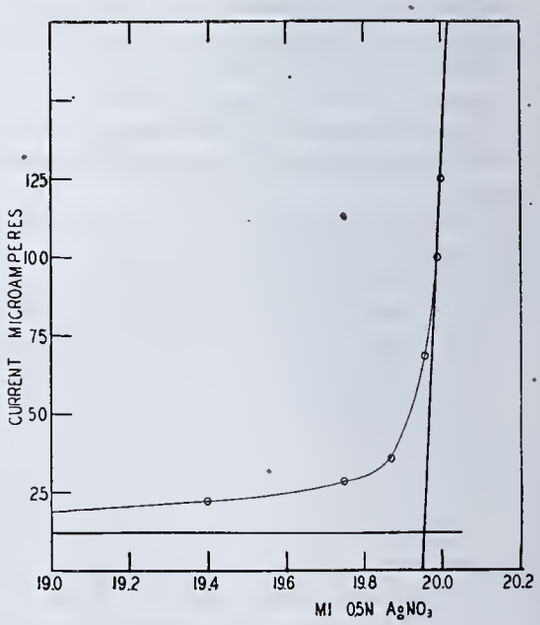

Figure 1. Titration Curve

100 ml. of 0.100 *N* NaCl, 0.8 *N* HNO_3, 0.1% gelatin, with 0.500 *N* $AgNO_3$

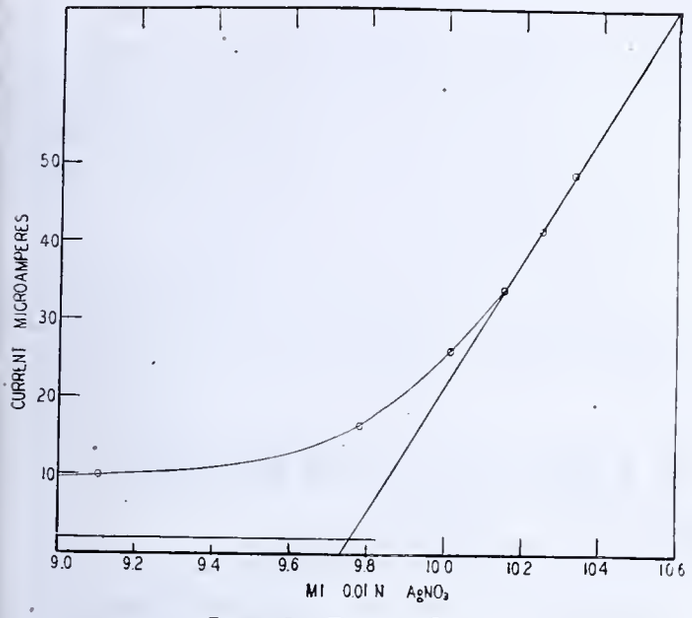

Figure 2. Titration Curve

100 ml. of 0.001 *N* NaCl, 0.8 *N* HNO_3, 0.1 % gelatin, in aqueous solution with 0.0100 *N* $AgNO_3$

ork showed that the addition of gelatin was unnecessary. The romide titration curves were similar in shape to those for chlo-de, except that a smaller current was observed before the end oint, allowing a higher galvanometer sensitivity to be used.

Pure potassium bromide was prepared by thermal decomposi-on of reagent quality potassium bromate in an electrically eated tube furnace at about 400°. Gravimetric standardization ieeked within 0.1% the normality of bromide solution prepared y using the potassium bromide as a primary standard substance.

Titration data for various concentrations of bromide in ueous solution and in 50% acetone using 0.8 *N* nitric acid and 1% gelatin are given in Table II. The results in general were mewhat low. In aqueous medium, 0.001 *N* bromide can be trated, while in 50% acetone, 0.0001 *N* bromide gave a 3% ror.

To prevent the interference of chloride, titrations in the pres-ce of 0.01 to 0.02 *N* ammonia were tried. It was found that 01 *N* ammonia could be added without affecting the bromide d point, using bromide concentrations as low as 0.001 *N*.

Table II. Titration of 100 Ml. of Bromide

ormality of Br^-	Normality of $AgNO_3$	Galvanometer Sensitivity	$AgNO_3$ Used Theory *Ml.*	$AgNO_3$ Used Experimental *Ml.*	Error %
		Aqueous Solution, 0.8 *N* Nitric Acid			
.009991	0.0500	1/20	19.98	19.80, 19.86 19.84, 19.92	−0.9, −0.6 −0.7, −0.3
.005115	0.0500	1/20	10.23	10.12, 10.13 10.08	−1.1, −1.0 −1.5
.002046	0.0100	1/20	20.46	19.96, 19.93 19.94	−2.5, −2.6 −2.6
.001023	0.0100	1/20	10.23	9.90, 9.96 9.88	−3.3, −2.7 −3.5
.000512	0.0100	1/20	5.12	4.91, 4.89 4.92	−4.1, −4.5 −3.9
		Silver Nitrate, 50% Acetone, 0.8 *N* Nitric Acid			
.01023	0.0500	1/20	20.46	20.21, 20.18 20.32, 20.15 20.36, 20.28	−1.2, −1.4 −0.7, −2.0 −0.5, −0.9
.005115	0.0500	1/20	10.23	10.17, 10.14 10.15, 10.05 10.09, 10.14	−0.6, −0.9 −0.8, −1.8 −1.4, −0.8
.002046	0.0100	1/20	20.46	19.95, 19.98 19.93, 19.95 20.11, 19.95	−2.6, −2.3 −2.7, −2.6 −1.8, −2.6
.001023	0.0100	1/20	10.23	9.88, 9.90 9.91, 9.92 9.92, 9.92	−3.5, −3.3 −3.2, −3.1 −3.1, −3.1
.000512	0.0100	1/20	5.12	4.98, 4.97 4.95	−2.7, −2.9 −3.3
.00010	0.0010	1/5	10.00	10.22, 10.26 10.03	+2.2, +2.6 +0.3

Later work showed that if bromide titrations are carried out in the absence of gelatin, chloride does not interfere, because a current is caused by silver chloride particles.

TITRATION OF IODIDE

A solution of sodium iodide was prepared from a Baker and Adamson reagent quality product and standardized by oxidation with nitrite, destruction of the excess nitrite with urea, and titration with thiosulfate.

The results of titrations in aqueous medium, 0.8 *N* nitric acid, and 0.1% gelatin are given in Table III. As with bromide, the addition of gelatin was found later to be unnecessary.

The effect of various concentrations of ammonia on the titration of iodide is shown in Table IV. With 0.01 *N* iodide, even 1 *N* ammonium hydroxide had no effect, while with 0.001 *N* iodide a 10% error was found using 0.5 *N* ammonia although 0.1 *N* ammonia had no effect. By adding 0.1 to 0.3 *N* ammonia, the interference of bromide and chloride can be prevented.

It is concluded that iodide can be successfully titrated in nitric acid, neutral, or dilute ammoniacal medium.

DISCUSSION

The amperometric method is useful for the rapid titration of halides in dilute solution and in the presence of large concentrations of acid or salt. A titration can be completed in 3 to 5 minutes, because the points of most interest on the titration curve are those lying beyond the end point. Although not a highly accurate method in dilute solution, results accurate to ±0.1% can be obtained in 0.1 *N* solutions.

Compared with the Mohr and Fajans procedures, the amperometric method has the advantages of applicability at high dilutions and in acid or alkaline solution. Compared with the Volhard method, it has the obvious advantage of a direct titration over a back-titration method.

Table III. Titration of 100 Ml. of Iodide

Normality of I^-	Normality of $AgNO_3$	Galvanometer Sensitivity	$AgNO_3$ Used Theory *Ml.*	$AgNO_3$ Used Experimental *Ml.*	Error %
		Silver Nitrate, Aqueous Solution, 0.8 *N* Nitric Acid			
0.0100	0.0500	1/20	20.00	19.81, 19.95 19.88, 19.91 19.96, 19.93 19.92	−0.9, −0.3 −0.6, −0.5 −0.2, −0.4 −0.4
0.00500	0.0500	1/20	10.00	9.97, 9.95 9.97	−0.3, −0.5 −0.3
0.00200	0.0100	1/10	20.00	19.77, 19.75 19.75	−1.1, −1.2 −1.2
0.00100	0.0100	1/10	10.00	9.83, 9.85 9.83	−1.7, −1.5 −1.7
0.00050	0.0100	1/10	5.00	4.88, 4.87 4.87	−2.4, −2.6 −2.6
0.000050	0.0010	1/2	5.00	4.35, 4.53 4.82	−13.0, −9.4 −3.6
0.000020	0.0010	1/2	2.00	1.77, 1.80 1.77	−11.5, −10.0 −11.5
Blank	0.0010	1/2	0.00	0.05	.....

Table IV. Titration of 100 Ml. of Iodide

Normality of I^-	Normality of $AgNO_3$	Normality of NH_4OH	Galvanometer Sensitivity	$AgNO_3$ Used *Ml.*	Error %
			Aqueous Medium, Various Concentrations of Ammonia		
0.0100	0.0500	0.01	1	19.64	−1.8
			1/2	19.78, 19.67	−1.1, −1.6
		0.02	1/2	19.60, 19.80 19.65	−2.0, −1.0 −1.8
		0.10	1/2	19.64, 19.63	−1.8, −1.9
		0.50	1/5	19.70, 19.58 19.72	−1.5, −2.1 −1.4
		1.0	1/2	19.64, 19.65	−1.8, −1.8
0.00100	0.0100	0.01	1/10	9.85, 9.86	−1.5, −1.4
			1/5	9.92, 9.89	−0.8, −1.1
		0.02	1/5	9.89, 9.84	−1.1, −1.6
		0.10	1/5	9.76, 9.84 9.86	−2.4, −1.6 −1.6
		0.50	1/2	9.01, 9.24	−9.9, −7.6
		1.0	1	8.44, 8.49	−15.6, −15.1

The potentiometric method can be applied in dilute solutions and in general is more accurate than the amperometric method, but is considerably slower. To obtain reliable results potentiometrically it is necessary that solubility equilibrium be reached at each point of the curve, especially in the near vicinity of the end point. By titrating to an equivalence potential, the potentiometric method can be made more rapid, but the attainment of equilibrium must be experimentally proved.

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Amperometric Titration of Mixtures of Halides Using the Rotating Platinum Electrode

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Iodide, bromide, and chloride can be successively titrated in mixtures with silver nitrate, using the rotating platinum electrode as an amperometric indicator electrode. Ammonia is added for the iodide titration, an excess of acid is added for the bromide titration, and gelatin is added for the chloride titration. Multivalent metallic ions added as flocculating agents do not appreciably affect the titration of bromide in bromide-chloride mixtures, indicating that mixed crystal equilibrium is not reached during the rapid addition of silver nitrate. The amperometric method is much more rapid than the potentiometric method for mixtures of halides, although not always as accurate.

CONSIDERABLE work has been done in the titration of mixtures of halides, especially by potentiometric titrations.

Bromide and iodide mixtures were first titrated potentiometrically by Behrend (*1*), who added sufficient ammonia to keep silver bromide in solution. He was unable to determine bromide in the presence of chloride. Dutoit and von Weisse (*4*) and Pinkhof (*14*) titrated iodide in the presence of bromide without adding ammonia.

In attempts to titrate bromide in the presence of chloride, Pinkhof (*14*) added ammonium carbonate to prevent the precipitation of silver chloride. By using the proper reference electrode, the titration was carried out until the bromide concentration was 10^{-3} *N*, and a correction of this amount was added to the result.

Liebich (*12*) attempted to titrate mixtures of halides. Titration of iodide gave high results in the presence of bromide, but accurate results were obtained in the presence of barium nitrate. Similar high results were found for iodide in the presence of chloride, and bromide in the presence of chloride. The addition of barium nitrate or alum was shown to improve the results. Clark (*2*, *3*) found about a 1% error in bromide titrations in the presence of an equal concentration of chloride in the presence of 5% barium nitrate. He also titrated mixtures of bromide and iodide in the presence of barium nitrate, and chloride-iodide mixtures without salt addition.

The high results obtained for the more insoluble halide in chloride-bromide or bromide-iodide mixtures have been attributed by Liebich (*12*) and Müller (*13*) to the formation of mixed crystals of silver halides as shown by Kuster (*10*) and Thiel (*16*) and studied in detail by Kolthoff and Eggertsen (*8*). Clark (*2*, *3*), however, considered adsorption to be responsible. Flood (*5*) calculated the theoretical error due to ideal mixed crystal formation assuming (*a*) that the entire precipitate is in equilibrium with the solution at all times and (*b*) that the precipitate coagulated upon formation, and only the part being precipitated at each instant is in mixed crystal equilibrium. Flood and Bruun (*6*) applied the results assuming "ideal inhomogeneous mixed crystal formation" (case *b*) to chloride-bromide mixtures and calculated correction factors to be applied to the results as a function of mole fraction of bromide in the mixture. Thus for an equimolar mixture, the bromide result should be 1.2% high and the chloride result an equal amount low. Flood and Bruun concluded that mixed crystal formation accounted largely for the experimental results but that adsorption also was a contributing factor. They suggested that increased dilution would diminish the error due to adsorption.

Zintl and Betz (*17*) titrated mixtures of halides in very dilute solution (about 0.001 *N*). Bromide could barely be titrated in the presence of 10 times as much chloride, iodide in the presence of 60 times as much bromide, and iodide in the presence of 500 times as much chloride. Flood and Sletten (*7*) and Schütz (*15*) worked out techniques for detecting potentiometric end points in chloride-bromide mixtures which require empirical corrections depending upon the composition of the mixture.

The potentiometric titration method suffers from the inherent difficulty that the potential break for bromide in the presence of chloride is only about 0.1 volt (*13*).

Table I. Effect of Multivalent Cations on Bromide Titration in Absence and Presence of Chloride

Normality of Br^-	Normality of Cl^-	Normality of $AgNO_3$	Electrolyte Present	$AgNO_3$ Used *Ml.*	Error %
0.01	...	0.1	.....	9.96, 9.90	−0.4, −1.0
			0.2 *M* Ba^{++}	9.95, 9.96, 9.97	−0.5, −0.4, −0
			0.005 *M* Al^{+++}	10.04, 9.96	0.4, −0.4
			0.005 *M* Al^{+++}, 0.8 *N* HNO_3	9.93, 9.94	−0.7, −0.6
			0.02 *M* Al^{+++}, 0.8 *N* HNO_3	10.00	0.0
			0.002 *M* Th^{+4}	10.05, 9.95	0.5, −0.5
0.001	...	0.01	.....	10.01	0.1
			0.002 *M* Th^{+4}	9.95, 10.04	−0.5, 0.4
0.01	0.1	0.1	0.8 *N* HNO_3	10.03, 10.03, 10.07	0.3, 0.3, 0.7
			0.2 *M* Ba^{++}	9.93, 9.92, 9.93	−0.7, −0.8, −0
			0.002 *M* Al^{+++}, 0.8 *N* HNO_3	10.03, 10.06	0.3, 0.6
			0.005 *M* Al^{+++}, 0.8 *N* HNO_3	10.02, 10.09	0.2, 0.9
			0.02 *M* Al^{+++}, 0.8 *N* HNO_3	10.00, 10.04	0.0, 0.5
			0.01 *M* Th^{+4}, 0.8 *N* HNO_3	10.05, 10.07	0.5, 0.7
			0.02 *M* Th^{+4}, 0.8 *N* HNO_3	10.07, 10.12, 10.12	0.7, 1.2, 1.2
0.01	0.02	0.1	0.8 *N* HNO_3	10.24, 10.20	2.4, 2.0
				10.08[a], 10.01[a]	0.8, 0.1
			0.2 *M* Ba^{++}, 0.8 *N* HNO_3	10.07, 10.06	0.7, 0.6
			0.02 *M* Al^{+++}	10.26, 10.22	2.6, 2.2
			0.02 *M* Th^{+4}	10.07, 10.12	0.7, 1.2
0.002	0.002	0.02	0.8 *N* HNO_3	9.71	−2.9
			0.2 *M* Ba^{++}	9.82, 9.58	−1.8, −4.2
			0.02 *M* Al^{+++}, 0.8 *N* HNO_3	9.43, 9.65	−5.7, −3.5

[a] 2 minutes stirring after each ml. of $AgNO_3$ added.

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It was to compare the amperometric and po-ntiometric end points as applied to the titra-on of halide mixtures that this study was ndertaken.

The amperometric titration of the individual alides has been described (*11*). Silver bromide nd silver iodide do not cathodically depolarize ne rotating platinum electrode. Silver chloride, owever, causes a cathodic current even in the resence of a large excess of chloride. Therefore is possible, in principle, to titrate either bromide iodide in the presence of chloride if no gelatin added to suppress the current due to silver loride. A chloride end point could then be btained by adding gelatin and shifting to a wer galvanometer sensitivity.

To determine iodide in the presence of bromide, ammonia can added to increase the solubility of silver bromide without fecting the iodide end point. Thus all three halides can be ccessively titrated by first adding ammonia for the iodide tration, then adding an excess of acid and titrating the bromide, d finally adding gelatin for the chloride end point.

EXPERIMENTAL

The effect of adding multivalent metallic ions as flocculating ents on the titration of bromide in the absence and presence of loride is shown in Table I. The apparatus and experimental ocedure have been described (*11*).

Although a trend toward higher results for bromide in the esence of increasing amounts of chloride is noticeable, the cculating ions in general had no effect on the end point. It possible that under the conditions of the amperometric titra-n in which the end point is rapidly passed (the entire titration xes only 3 to 5 minutes), mixed crystal equilibrium is not ached even though the precipitate is colloidally dispersed. two experiments (Table I), the titration was delayed for 2 nutes after the addition of each milliliter of reagent. Con-ry to expectation, the results were lower than for the same tration run rapidly. The presence of five or ten times as much loride as bromide led to a flat titration curve with no bromide d point. For solutions less than 0.002 *N* in both bromide and loride, low results were obtained for bromide. At higher con-ntrations, a balancing of errors occurs, the "normal" low result the amperometric bromide titration being balanced by a sitive error caused by mixed crystal formation.

In Table II, data are given for several titrations of iodide-omide mixtures in the presence of various concentrations of monia. It appears that 0.2 *N* ammonia is too concentrated solutions of iodide more dilute than 0.01 *N*. In general, a ncentration of 0.1 *N* ammonia is suitable for all but the most ite iodide solutions.

Table II. Titration of Iodide in Presence of Bromide in Ammoniacal Solution

or-rality f I^-	Nor-mality of Br^-	Nor-mality of $AgNO_3$	Nor-mality of NH_3	$AgNO_3$ Used *Ml.*	Error %
0 1	...	0.1	..	9.94	−0.6
0 1	0.01	0.1	0.1	9.94, 9.93, 9.94	−0.6, −0.7, −0.6
			0.2	9.90, 9.96, 9.92	−1.0, −0.4, −0.8
0 1	0.05	0.1	0.2	9.88, 9.83, 9.82	−1.2, −1.7, −1.8
0 01	0.001	0.01	0.1	9.99, 9.93	−0.1, −0.7
			0.2	9.64, 9.58	−3.6, −4.2
0 01	0.01	0.01	0.1	9.80, 9.80, 9.88[a]	−2.0, −2.0, −1.2
0 001	0.0001	0.001	0.05	10.07, 9.95	0.7, −0.5
			0 1	9.46, 9.31[a]	−5.4, −6.9
			0.2	8.77[a]	−12.3

[a] 0.1% gelatin added.

Table III. Titration of Mixtures of Iodide, Bromide, and Chloride

Sample	Normality of Halide	Normality of $AgNO_3$	Medium	Galvanometer Sensitivity	$AgNO_3$ Used *Ml.*	Error %
1	0.01 *N* I^-	0.1	0.1 *N* NH_3	1/2	9.97, 9.94	−0.3, −0.6
	0.01 *N* Br^-	0.1	0.8 *N* HNO_3	1/5	10.10, 10.01	1.0, 0.1
	0.01 *N* Cl^-	0.1	0.1 % gel.	1/20	9.86, 9.73	−1.4, −2.71
2	0.18 *N* I^-	0.1	0.1 *N* NH_3	1/2	17.91	−0.5
	0.009 *N* Br^-	0.1	0.8 *N* HNO_3	1/5	8.92, 8.81	−0.9, −2.1
	0.003 *N* Cl^-	0.05	0.1 % gel.	1/20	6.06, 6.01	1.2, 0.2
3	0.003 *N* I^-	0.05	0.1 *N* NH_3	1/2	5.86, 5.93	−2.3, −1.2
	0.018 *N* Br^-	0.1	0.8 *N* HNO_3	1/5	18.10, 18.12	0.3, 0.6
	0.009 *N* Cl^-	0.1	0.1 % gel.	1/20	8.91	−1.0
4	0.009 *N* I^-	0.1	0.1 *N* NH_3	1/2	8.98	−0.2
	0.003 *N* Br^-	0.05	0.8 *N* HNO_3	1/5	6.07	1.2
	0.018 *N* Cl^-	0.1	0.1 % gel.	1/20	17.75	−1.4

The results obtained in titrations of all three halides are shown in Table III. As indicated, a 0.1 *N* ammonia medium was used for the iodide titration, followed by 0.8 *N* ammonia for the bromide and 0.1% gelatin for the chloride. The galvanometer sensitivity of about 0.01 microampere per mm. was cut by means of an Ayrton shunt to 1/2 for the iodide, 1/5 for the bromide, and 1/20 for the chloride titration to obtain a suitable residual current line in each titration. As a reference electrode, the mercury-mercuric iodide–potassium iodide cell of Kolthoff and Harris (*9*, potential −0.23 volt *vs.* saturated calomel electrode) was used for the iodide titration in ammoniacal medium, followed by a saturated calomel reference electrode for the bromide and chloride titrations.

No difficulty was encountered except in the bromide titration of sample 4, when only about 10% of the total halide present was bromide. In this case, the bromide curve was flat with an indistinct end point.

In Table IV, data are shown for the analysis of three synthetic halide mixtures of varying composition, made by one worker as unknowns and analyzed by another.

The amperometric method shows promise as a rapid method for the determination of halides in mixtures, although it is not always as accurate as the slower potentiometric method.

Table IV. Analysis of Unknown Halide Mixtures

Sample	Present I^-	Present Br^-	Present Cl^-	Found I^-	Found Br^-	Found Cl^-
	Mg./100 ml.			*Mg./100 ml.*		
1	15.15	155.1	0.43	15.0	153.8	0.62
2	2.54	73.5	312.0	2.0	76.2	305.6
3	121.8	17.6	21.3	121.3	15.3	21.4

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Centrifugal Sedimentation Method for Particle Size Distribution[1]

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The centrifugal sedimentation method for particle size distribution of materials in a dispersed system is reviewed. A preferred method has been used which is analogous to Odén's method of tangential intercepts for gravitational sedimentation. This procedure yields the same results as those obtained by the variable suspension height method but is more convenient from the practical point of view. Examples are given to show practical applications of the beaker-type centrifuge to the study of relative dispersion of titanium dioxide pigment in paint systems. The method is of limited usefulness for the determination of specific surface area or the relative efficiency of light-diffusing properties of materials which are either aggregated or irregularly shaped, since Stokes' law is based on the assumption of an equivalent spherical particle. Electron micrographs supplement the sedimentation studies.

THE determination of the particle size distribution of finely divided material is of practical and fundamental importance in physicochemical studies. In particular, centrifugal sedimentation has proved very valuable in determining the relative distribution of pigments in paint vehicles (*7*). The theory underlying the application of sedimentation data for the calculation of the particle size distribution has been worked out (*3*, *8*, *9*) and verified by comparison with the results obtained by other methods (*4*, *5*).

From the practical point of view the use of a suitable dispersion technique for the particulate material is of significance, since the sedimentation data are of little value unless a satisfactory dispersion has been attained. Hence, cognizance must be taken of the nature of the dispersing agent used, and the particular technique employed in preparing the dispersion. A further limitation is the effect of particle shape. The concept of "equivalent spherical diameter" has been used in all applications of Stokes' law, but it is known from electron microscope studies that the particles are rarely spherical, often being irregular aggregates of primary particles. In extreme conditions poor correlation may exist between specific surface as calculated from particle size distribution data and specific surface as determined by independent methods.

THEORETICAL

The gravitational method of sedimentation has proved suitable for determining the particle size distribution of particles in suspensions where the size range is from about 1 to 50 microns. The procedure used involves the application of Stokes' law (*4*, *5*, *11*) and Odén's method of tangential intercepts (*8*, *9*).

The equating of the frictional force, as given by Stokes' law, to the gravitational force on the particle yields the relation

$$D = \sqrt{\frac{18\eta h}{(d_1 - d_0)gt}} \qquad (1)$$

where D = equivalent spherical diameter of the particle
η = coefficient of viscosity of the suspension medium
h = height of suspension
d_1 = density of particle
d_0 = density of suspension medium
g = gravitational acceleration
t = time of settling

Since the usual method of obtaining data on a polydisperse suspension is to determine the sedimentation-time curve, it is apparent that in time t, all particles having a diameter equal to o greater than D_m will settle through height h. In addition, a certain fraction of the particles having a diameter less than D_m wil settle through a height less than h. The deduction of the sedimented "fines" may be accomplished by Odén's method of tangential intercepts in which

$$\int_0^{D_m} F(D)dD = (1 - p) + t\frac{dp}{dt} \qquad (2$$

where p = total weight fraction sedimented
$F(D)dD$ = weight fraction of particles having a diameter between D and $D + dD$

For materials with a particle size range of approximately 0. to 1 micron, the ordinary beaker-type centrifuge has been employed to determine the distribution. The essential point of divergence from gravitational sedimentation is that the force actin on the particle increases with the settling distance. As firs shown by Svedberg and Nichols (*12*) the gravitational equatio must then be modified to

$$D = \sqrt{\frac{18\eta \ln x_2/x_1}{(d_1 - d_0)\omega^2 t}} \qquad (3$$

where x_1 = distance from the center of rotation to a point in th suspension
x_2 = distance from the center of rotation to a second poir in the suspension
ω = angular velocity in radians per second

The calculation of the particle size distribution from the sedimentation-time curve was apparently first accomplished by Ron walter and Vendl (*10*) who arrived at the equation

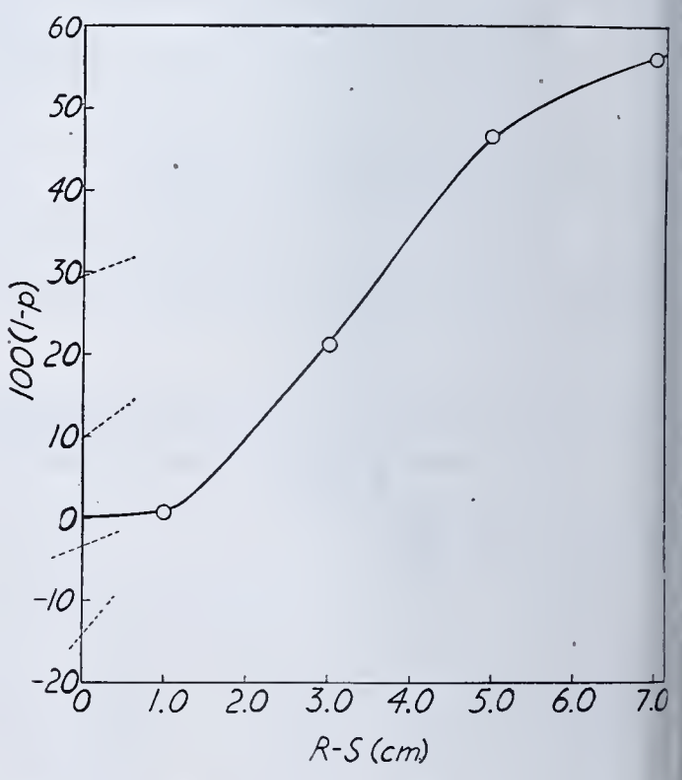

Figure 1. Sedimentation of Titanium Dioxide in Dutch Oil Z2

Variable suspension height method

[1] Three papers presented in the Symposium on Measurement and Creation of Particle Size at the Twelfth Annual Chemical Engineering Symposium of the Division of Industrial and Engineering Chemistry are presented here, pages 360–73. Other papers in the symposium will be published in the July issue of the INDUSTRIAL EDITION.

$$\int_0^{D_m} F(D)dD = \frac{R^2 - S^2}{S^2} \frac{1}{\ln R^2/S^2} t \frac{dp}{dt} \quad (4)$$

ere R = distance from center of rotation to bottom of cup
S = distance from center of rotation to surface of suspension

Iowever, this equation was shown by Brown (*3*) to be invalid l this conclusion has been confirmed by the authors. Thus, ce R and S are constants, the weight fraction of particles hav- diameters between 0 and D_m is given by

$$\int_0^{D_m} F(D)dD = K \times t \frac{dp}{dt} \quad (4')$$

herefore the value of the integral must run from 0 to 1 as the ticle diameter is increased from 0 to ∞. Direct application the sedimentation-time curve, however, shows that the term $\frac{dp}{dt}$ and hence the integral $\int_0^{D_m} F(D)dD$ go through a maxi-

m, since at low values of t, $t \frac{dp}{dt}$ equals a small number and, at h values of t, it is again a small number since the slope $\frac{dp}{dt}$ ap- aches 0.

rown was unable to find a mathematical solution for the dis- ution function $F(D)$ when the time of centrifuging was used as ariable, but did arrive at a satisfactory solution when the sus- sion height $(R - S)$ was varied. His equation for cylindrical trifuge tubes is:

$$\int_0^{D_m} F(D)dD = (1 - p) + (R - S) \frac{dp}{dS} \quad (5)$$

his equation has been used by the authors, and results have n expressed as a cumulative curve, $\int_0^{D_m} F(D)dD$ *vs.* D_m er than as the distribution function itself, $F(D)$ *vs.* D_m, since latter would involve second derivatives, a procedure which is ably not justified by the experimental accuracy achieved.

n actual practice, however, it has been found somewhat more venient to use a modified method for the determination of the ulative weight per cent curve. The principle of this method, ch has already been pointed out in the work of both Brown (*3*) Martin (*7*), is that centrifugal sedimentation can be made to oach gravitational sedimentation by making the height of ension $(R - S)$ small. This means that the centrifugal force he particle is taken to be approximately constant over the ing distance. Hence, the mathematical procedure is identical the gravitational calculations, except that the gravitational leration, g, is replaced by the centrifugal acceleration, $\omega^2 R$. results obtained by this method have been found to check ely those obtained by the variable suspension height method many pigment dispersions.

here are two reasons for preferring to use a small height of ension with variable time rather than variable suspension ht and constant time. First, the former method requires iderably less sample, which is an important factor where ed quantities are available and, secondly, a wider particle range can be covered without having to readjust the centri- conditions. Thus, using the variable suspension height pro- re, two or more analyses may be necessary at different centri- speeds in order to cover adequately the particle size range lly encountered. From the physical viewpoint the require- of sector-shaped tubes is also more closely approached when suspension height is made small. On the negative side it be considered that the relative experimental error involved easuring the height is larger when the height is small. How- by carefully calibrating the centrifuge cups on a volume , the error seems to have been minimized as judged by the

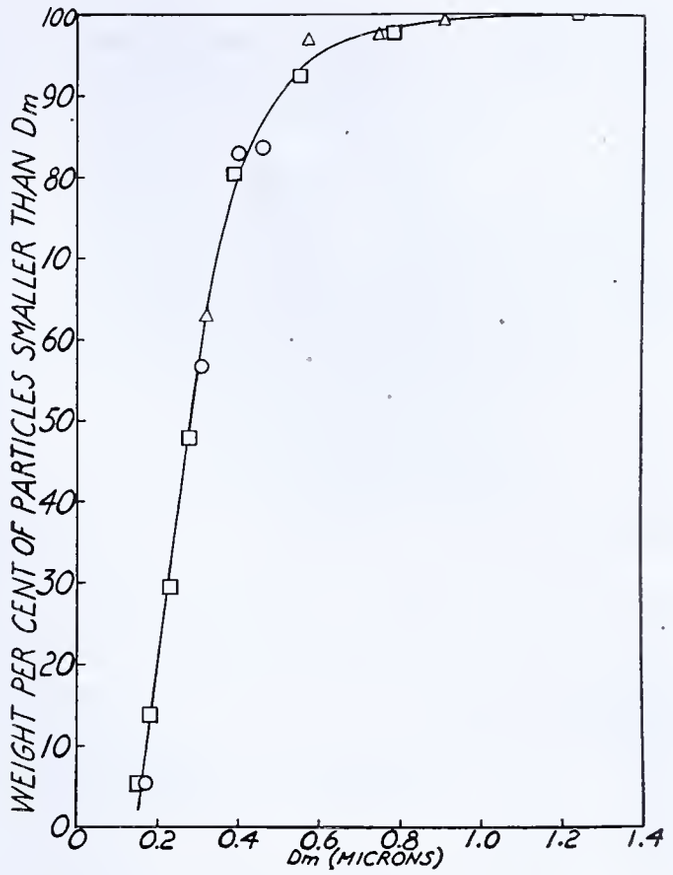

Figure 2. Cumulative Weight Per Cent Curve for Titanium Dioxide in Dutch Oil Z2

○ Variable suspension height method, 650 r.p.m.
△ Variable suspension height method, 350 r.p.m.
□ Modified gravitational method

reproducibility of the results, and their agreement with other methods.

EXPERIMENTAL

Apparatus. The apparatus used was an International centrifuge, size 1, Type SB, equipped with 115-volt Transtat voltage regulator which was designed to improve the constancy of the rotational speed. The centrifuge head holds eight chromium-plated cylindrical cups (9.20 × 3.58 cm.) each having a cover to prevent solvent evaporation. The distance, R, from the center of rotation to the bottom of the centrifuge cup was 21.3 cm. The centrifuge speed was measured with a General Radio Strobotac and the time of acceleration and deceleration allowed for by the method of Marshall (*6*). For the centrifuge speed used in the experiments described, the correction was one minute.

The pigment dispersions were prepared by mixing titanium dioxide with an organic vehicle in the ratio of 100 to 54 grams in a mechanical mixer for 60 minutes. The mixture was then put through a three-roll laboratory paint mill with the rolls set at 0.075 mm. (0.003 inch) and finally diluted to approximately 3% solids with mineral spirits. The pigment suspension was analyzed by pipetting a 10-ml. aliquot into a weighed crucible, burning off the organic matter, and weighing the ignited residue as titanium dioxide. In the case of the centrifuged samples the supernatant suspension was carefully separated from the sedimented material and mixed before sampling. Since the suspension is dilute, the per cent sedimented may be calculated in the following manner:

$$\% \text{ sedimented} = 100p = \frac{\text{weight of sediment}}{\text{weight of total}} \times 100 =$$

$$\frac{\text{weight of total} - \text{weight in suspension}}{\text{weight of total}} \times 100$$

$$\approx \frac{(C_{TiO_2})_{\text{initial}} - (C_{TiO_2})_{\text{suspension}}}{(C_{TiO_2})_{\text{initial}}} \times 100$$

where (C_{TiO_2}) = concentration of TiO_2 in grams per liter.

Comparisons of Methods. In applying the variable suspension height method it was found convenient to modify Equation 5 to

Table I. Sedimentation of Titanium Dioxide

$R - S$	$100 \times (1 - p)$	$2 \times 100(1 - p)$	Tangential Intercept[a]	$100 \int_0^{D_m} F(D)dD$	D_m[b]
Cm.	%	%	%	%	μ
1.00	0.94	1.9	− 3.5	5.4	0.17
3.00	21.1	42.2	−14.5	56.7	0.30
5.00	46.6	93.2	10.0	83.0	0.40
7.00	56.0	112.0	29.5	83.5	0.46

[a] Values taken from Figure 1.
[b] From Equation 3, using $X_1 = S$, $X_2 = R$, $d = 4.20$ grams per cc., $d_0 = 0.80$ grams per cc. and $\eta = 0.0096$ poise at 25° C. we have:

$$D_m = \sqrt{\frac{18 \times 0.0096 \times 2.303 \log \frac{21.3}{21.3 - (R - S)}}{(4.20 - 0.80)\left(\frac{2\pi \times 650}{60}\right)^2 30 \times 60}}$$

$$D_m = \sqrt{1.400 \log \frac{21.3}{21.3 - (R - S)}} \text{ microns}$$

$$\int_0^{D_m} F(D)dD = 2(1 - p) - \text{tangential intercept} \qquad (5')$$

where the tangential intercept is that obtained from the $100(1 - p)$ versus $(R - S)$ curve.

The results summarized in Table I were obtained using a centrifuge speed of 650 r.p.m. with a time of 30 minutes. The dispersion medium was a linseed oil vehicle (National Lead Company, Dutch Oil Z2).

These results, however, cover only the particle size interval from 0.17 to 0.46 micron and leave an appreciable fraction of the distribution unaccounted for. Hence, with the variable suspension height method, a second analysis was necessary using different conditions—viz., 350 r.p.m. and a time of 30 minutes. The end results are expressed together with those of Table I in the form of a cumulative weight per cent curve (Figure 2).

The modified gravitational method, using $(R - S) = 1$ cm. was carried out with the previous suspension, so that a comparis between the two methods might be made. In order to apply t intercept method, Equation 2 was changed to

$$\int_0^{D_m} F(D)dD = 1 - \text{tangential intercept} \qquad (2$$

and Equation 1 to

$$D_m = \sqrt{\frac{18\eta(R - S)}{(d_1 - d_0)\omega^2 Rt}} \qquad (1$$

The results are summarized in Table II.

The cumulative weight per cent curve is also shown in Figure The close agreement with the variable suspension height meth is evident. It should be mentioned in connection with the pre sion of the modified gravitational method that a check analy on a portion of the original suspension showed an absolute er not greater than 1%.

Figure 4. Electron Micrograph of Titanium Dioxide, × 15,000

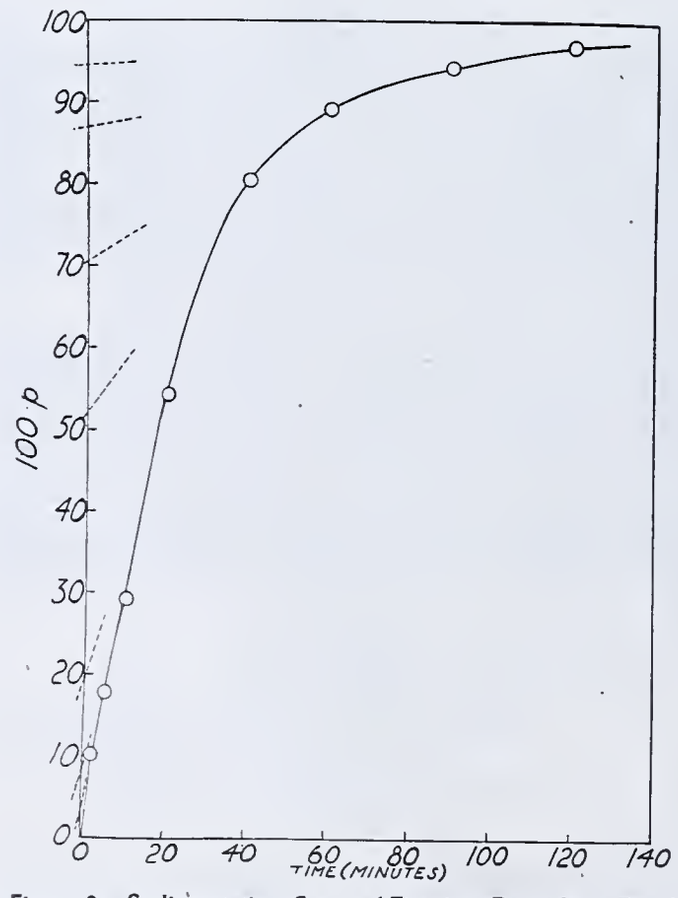

Figure 3. Sedimentation Curve of Titanium Dioxide in Dutch Oil Z2

Modified gravitational method

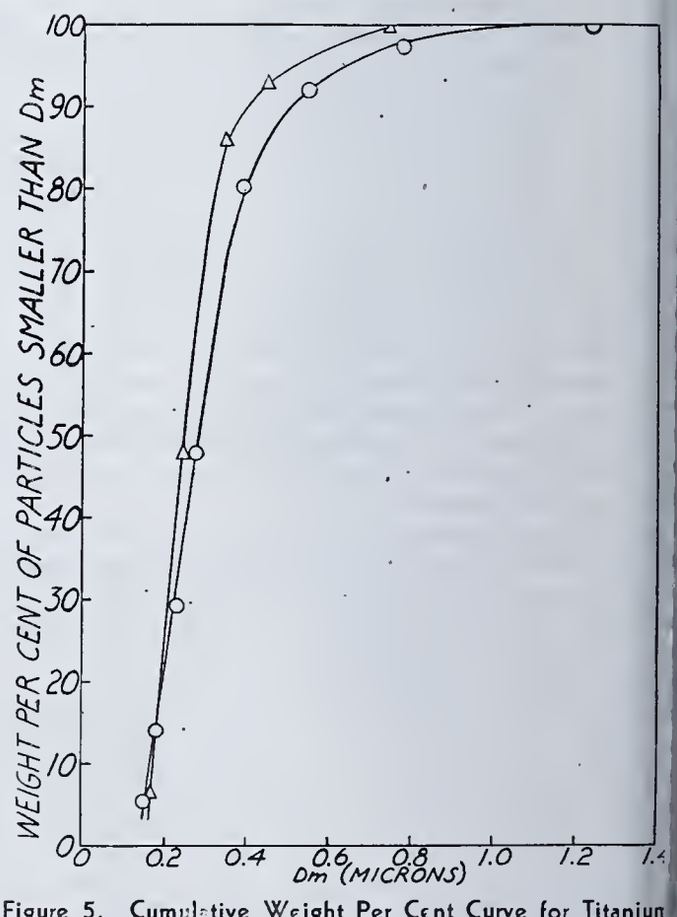

Figure 5. Cumulative Weight Per Cent Curve for Titanium Dioxide in Dutch Oil Z2

○ Modified gravitational method
△ Electron micrograph count method

Table II. Sedimentation of Titanium Dioxide

Time	$100 \times p$	Tangential Intercept[a]	$100 \int_0^{D_m} F(D)dD$	D_m[b]
Min.	%	%	%	μ
2	10.3	0	100	1.24
5	17.8	2.5	97.5	0.78
10	29.2	7.5	92.5	0.55
20	54.3	19.5	80.5	0.39
40	80.8	52.0	48.0	0.28
60	89.0	70.5	29.5	0.23
90	94.5	87.0	13.0	0.18
120	97.0	94.5	5.5	0.15

[a] Values taken from Figure 3.
[b] From Equation 1′ using $\eta = 0.0096$ poise, $(R - S) = 1$ cm., $d_1 = 4.20$ ms per cc., $d_0 = 0.80$ gram per cc., $R = 20.8$ cm., $\omega = \frac{2\pi \times 350}{60}$ radians per nd.

Table III. Effect of Milling

Milled		Not Milled	
$100 \int_0^{D_m} F(D)dD$	D_m	$100 \int_0^{D_m} F(D)dD$	D_m
%	μ	%	μ
14.3	0.20	5.6	0.20
45.0	0.30	29.2	0.30
67.2	0.40	55.7	0.40
81.0	0.50	75.0	0.50
96.7	1.00	95.0	1.00

lthough the agreement between the two methods lends lence to the fundamental "correctness" of the distributions ved at by means of the centrifuge, it was thought advisable heck the results by an independent method. A series of tron micrographs was taken of the pigment from which a ticle size distribution was made by the count method (*1*). r 500 particles were counted. The specimens for the electron roscope were made by incorporating a small amount of the inal paint with collodion which was used as a supporting film. ypical micrograph is shown in Figure 4. The particle size ribution is represented graphically in Figure 5 for comparison h the centrifuge data. The comparative results are in reason- agreement, considering the divergence in technique.

PRACTICAL APPLICATIONS

FFECT OF MILLING. To illustrate that the method of prepar- the dispersion is of fundamental importance and has to be sidered with any data given on particle size distribution, two ersion procedures were used for the same pigment. In both s a 60-minute mix was carried out in a small mechanical mixer g a linseed oil–tung oil vehicle (known to the paint trade as -1215, which was developed by the Technical Service oratories of the Titanium Pigment Corp., New York, N. Y.). half of the pigment paste was subsequently put through the ratory paint mill and the other half was

The cumulative weight per cent distribu- s, as determined by the modified gravita- al method, are given in Table III. These a show that milling has the effect of pro- ng a finer particle size d stribution.

ATURE OF DISPERSING VEHICLE. Since in practical preparation of paint dispersions, de variety of vehicles are available, the ibution obtained will depend also on the cle used. As an example another titanium ide pigment was dispersed according to previously described procedure in both a ch Oil Z2 and a linseed oil–tung oil vehicle -1215). The cumulative weight per cent ibutions, as determined by the modified itational method, are given in Table IV. ese data again emphasize the relationship een the particle size distribution and the ersion conditions.

INFLUENCE OF PARTICLE SHAPE. Electron micrograph studies of various titanium dioxide pigments have shown that the pigment particles are invariably aggregates of primary particles. As an extreme case it is possible to have approximately the same particle size distribution for two different pigments, one of which is composed of aggregates having very small primary particles and the other composed of aggregates having large primary particles. The cumulative weight per cent distributions, using a linseed oil–tung oil vehicle (VM-1215), are summarized in Table V.

Electron micrographs (Figure 6) indicate that the particulate material in the pigment composed of small primary particles has a more open structure and hence considerably more specific surface.

The particle shape may also be a factor where the number of particles in an aggregate is approximately the same, but the shapes of the primary particles differ widely. The following example shows the cumulative weight per cent distribution for a pigment composed of acicular primary particles and one having approximately spherical particles. The vehicle is again VM-1215.

The corresponding electron micrographs, as shown in Figure 7, illustrate the difference in particle shape. Surprisingly enough, those pigments have approximately the same hiding power (*2*). It is therefore apparent that complete reliance on sedimentation data would again lead to error in the evaluation of these products.

CORRELATION WITH OTHER PROPERTIES

SPECIFIC SURFACE. An estimate of the specific surface from a study of electron micrographs in comparison with the specific

Table IV. Effect of Dispersing Vehicle

Dutch Oil Z2		Linseed Oil–Tung Oil (VM-1215)	
$100 \int_0^{D_m} F(D)dD$	D_m	$100 \int_0^{D_m} F(D)dD$	D_m
%	μ	%	μ
12.0	0.15	3.0	0.15
41.5	0.20	20.0	0.20
71.5	0.30	51.5	0.30
87.0	0.40	72.5	0.40
93.0	0.50	84.5	0.50
100.0	1.00	97.5	1.00

Table V. Influence of Particle Shape

Large Primary Particles		Small Primary Particles	
$100 \int_0^{D_m} F(D)dD$	D_m	$100 \int_0^{D_m} F(D)dD$	D_m
%	μ	%	μ
11.2	0.20	13.1	0.20
42.1	0.30	38.2	0.30
59.9	0.40	59.1	0.40
75.9	0.50	69.7	0.50
92.8	1.00	90.8	1.00

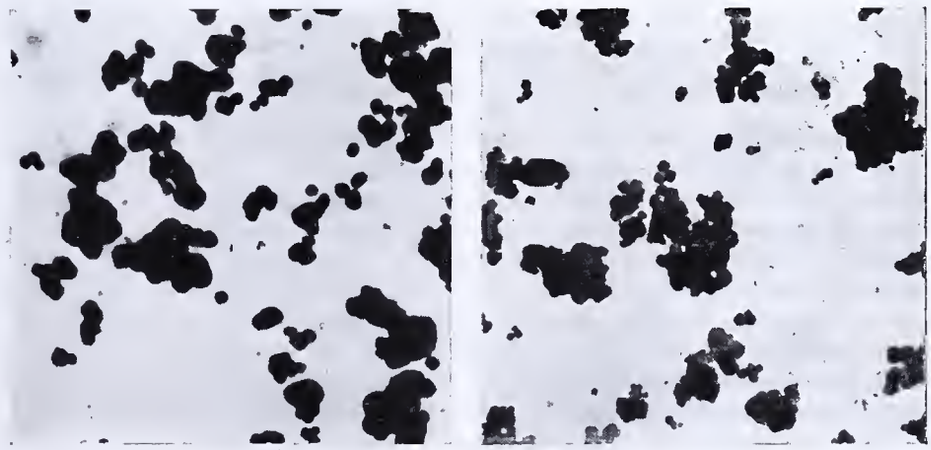

Figure 6. Electron Micrographs of Titanium Dioxide, ×15,000

Left. Large primary particles. *Right.* Small primary particles

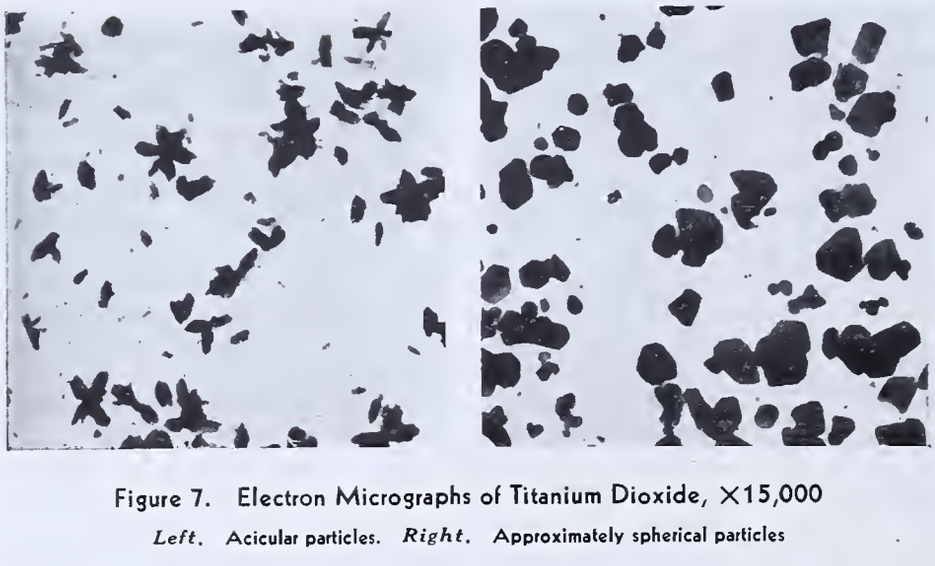

Figure 7. Electron Micrographs of Titanium Dioxide, ×15,000

Left. Acicular particles. *Right.* Approximately spherical particles

SUMMARY

The centrifugal sedimentation method based on proved fundamental principles, t making it suitable for determining cumula particle size distribution of particulate mate in dilute suspensions.

A modified centrifugal sedimentation p cedure has been employed in which the a lytical results agree with the results obtai by the variable suspension height method with electron micrographic count.

Examples have been presented to show practical use of the method for physicochem studies dealing with the dispersion of titan dioxide pigments in organic vehicles.

Table VI. Influence of Particle Shape

Acicular $100\int_0^{D_m} F(dD)D$ %	Acicular D_m μ	Approximately Spherical $100\int_0^{D_m} F(D)dD$ %	Approximately Spherical D_m μ
30.0	0.20	9.7	0.20
49.3	0.30	62.7	0.30
67.1	0.40	81.0	0.40
73.0	0.50	86.8	0.50
88.7	1.00	98.0	1.00

Table VII. Specific Surface

	Large Primary Particles	Small Primary Particles
	Sq. m. per gram	
Centrifugal sedimentation method	4.7	4.8
Electron micrograph method	5	10

Figure 8. Electron Micrograph of Titanium Dioxide, ×15,000

Small primary particles, ground in agate mortar

surface calculated from centrifugal sedimentation data is given in Table VII. In the case of the former a particle size distribution by the count method was made considering as far as possible only the primary particles. Figure 8, which is a second electron micrograph of the pigment shown in Figure 6 (right), was used for the count. This differs from the latter figure in that the dry pigment was vigorously ground in an agate mortar in order to deaggregate it as much as possible. The equation employed for the determination of specific surface from size distribution data is

$$S = \frac{6}{100d}\sum\frac{W\%}{D}$$

where $W\%$ = per cent by weight of the total pigment per given fraction
D = mean diameter for the size intervals in microns
Σ = summation of fractions in the size distribution
d = density in grams per cc.
S = specific surface in square meters per gram

A limitation of the centrifugal method as far as disclosing the inherent nature of the material is clearly indicated here.

Hiding Power. From a practical point of view, aggregate shape and size are important factors involved in the light-diffusing properties of paints. A measure of this property can be made by determining the hiding power of the paint. Referring to the pair of paints on which data have been presented in Table V (which for all practical purposes show close agreement), the pigment with large primary particles has 10% greater hiding power than the pigment in which the particles are composed of small primary particles. On this basis it would be a fallacy to rely completely on sedimentation studies for depicting light-diffusing qualities of particulate materials.

Limitations of the sedimentation method for the determina of specific surface area and the hiding power of particu material which are aggregated or nonspherical in shape h been emphasized.

ACKNOWLEDGMENT

Appreciation is expressed to R. Dahlstrom, director of resea National Lead Company, Titanium Division, for his interest suggestions.

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Particle Size by Spectral Transmission

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imple method has been developed for determining two-param- size distributions from transmissions measured in the visible near infrared parts of the spectrum. It is based on an empirical ionship between scattering, particle size, refractive index, and wave length of light. The method was developed for essentially absorbing materials and was originally based on particle size surements made with the low-speed Svedberg ultracentrifuge.

HE spectral transmission method of particle size analysis is a echnique for obtaining information on the particle size dis- utions of finely divided colorless powders from the amount of they transmit in completely deflocculated dilute suspen- s. Usually the only data required are a series of transmis- s measured over a spectral range of 0.4 to 2.0 microns. The hod is limited to colorless materials and to particle sizes for h optimum scattering occurs in the range over which the smissions can be measured.

ght scattering has been used in the study of particle size since leigh (*7*) did his fundamental work nearly 50 years ago. d (*6*) and Gamble and Barnett (*3*) have made use of Ray- 's equation in the study of comparatively large particles by ying out their measurements in the near infrared. Stratton Houghton (*8*), and very recently Barnes and La Mer (*2*), described theoretical curves which apply to scattering sys- s when the particle size is of the same order as the wave th of the radiation. Both of these curves are based on the tions of Mie (*5*) and apply only to a specific value of the rela- refractive index of the particle. The basis for the present icle size method is a more general empirical relationship h was derived during studies on scattering systems carried with a low-speed Svedberg ultracentrifuge (*9*). A brief de- tion of the basic relationship is given below to aid in the dis- on of the method of particle size analysis and a more com- description of the work on scattering systems anned for later publication.

DERIVATION OF SCATTERING FUNCTION

far as possible the terms used in the following ission are defined according to established ention.

ecific extinctions are calculated from the ured transmissions of scattering suspensions ding to the Beer-Lambert law as illustrated quation 1

$$\frac{I}{I_0} = e^{-\bar{E}_M ct} \qquad (1)$$

e I is the intensity of the transmitted light; I_0, tensity of the incident light; $\bar{E}_M$, the average fic extinction for the particle size distribution ved; the subscript, M, refers to a function mined by the relative refractive index of the cle and the wave length of the radiation used; e volume concentration of the suspension in of 0.01 cc. of suspended material per 100 cc. spension; and t, the cell thickness in centi- rs. Particle size is measured as particle radius he unit used for both particle radius and wave h of radiation is 1 micron. One micron is 10^{-4} r 10^4 Ångström units. Relative refractive index e refractive index of the particle divided by efractive index of the suspension medium. e distributions used the areas under the distribution curve correspond to the volume of material in a given size range and not to the number of particles.

The primary variables which determine specific extinction in a scattering system are the size and relative refractive index of the suspended particles and the wave length of the incident radiation. These variables were studied by fractionating four nearly colorless minerals covering a refractive index range of 1.36 to 2.45 into 6 to 9 relatively homogeneous samples. The particle size distributions and the specific extinctions of each of the fractionated samples were determined at the same time in a Svedberg low-speed untracentrifuge, in a suspension medium whose refractive index was 1.47. The radiation used covered a band of wave lengths from 0.400 to 0.480 micron and the average wave length was calculated to be 0.450 micron.

The four curves shown in Figure 1, in which specific extinction is plotted as function of particle size, were then derived from these data. In this step a set of simultaneous integral equations was solved for each group of samples with an instrument called the moment-product integrator (*9*). When the coordinates of Figure 1 were transformed, so that the product of specific extinctions and radius were plotted against the product of the Lorentz refractive index coefficient, $\frac{m^2 - 1}{m^2 + 2}$, and the radius, the four curves were very nearly superimposed. In the refractive index term m is the relative refractive index. A comparison of measured and calculated transmissions over the wave-length range of 0.7 to 2.0 microns showed that if the abscissa unit were assumed to be $\left[\frac{m^2 - 1}{m^2 + 2}\right]\frac{n_0}{\lambda}$ multiplied by the radius, a satisfactory agreement was obtained. In the final term n_0 is the refractive index of the suspension medium and λ is the wave length of the radiation in air. For convenience the factor which modifies the particle radius has been called M.

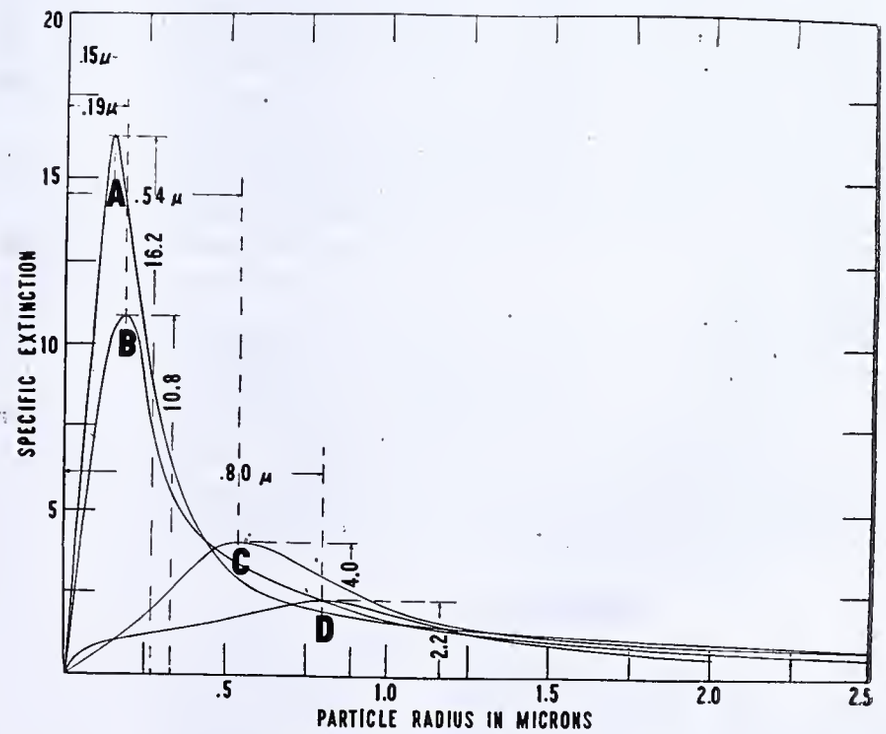

Figure 1. Mineral Series

A. Sphalerite, n = 2.47
B. Cerussite, n = 2.03
C. Barytes, n = 1.66
D. Cryolite, n = 1.37
λ = 0.450μ

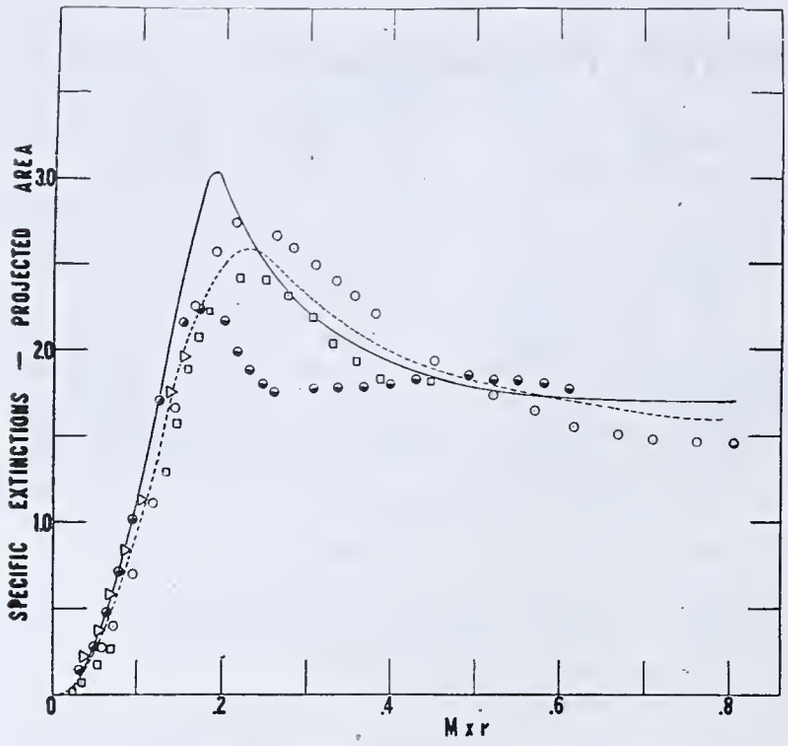

Figure 2. Scattering Function

———Adjusted for monochromatic light
- - - - -Original function
⊙ Sphalerite. ◒ Cerussite. △ Cryolite. ⊡ Barytes.

Figure 2 shows the data plotted on the new basis. The dotted curve shows the original form of the curve and the solid curve shows the form adopted after new measurements had been made and corrections had been applied to compensate for the use of heterochromatic light in obtaining the original data (*1*). In Figure 2 the specific extinctions are no longer on a volume basis but on a basis which corresponds to the volume of particles divided by the particle radius and called specific extinction per unit projected area. The spread of the points near the maximum is probably due to the indirect method which had to be used in constructing the curves of Figure 1. The deviation of the cerussite curve from the barytes and sphalerite curves is due largely to the double refraction of this mineral. The agreement between specific extinctions calculated from the solid curve of Figure 2 with measured values over a wide range of M and particle radius values shows that the curve gives a good approximation of the scattering behavior of systems of transparent particles. The minimum and second maximum shown in the corresponding curves of Stratton and Houghton, and Barnes and La Mer were not found. These features may have been obscured by the lack of homogeneity of the fractions at large values of Mr or by the departure from spherical shape of the particles used in the derivation. In any event, the form of the curve shown in Figure 2 has proved sufficiently accurate for the analysis of a wide variety of practical systems.

The scattering function shown in Figure 2 makes possible the calculation of specific extinction as a function of radius for values of M corresponding to a range of relative refractive indices from 1 to 2, for wave lengths from 0.4 to 2.0 microns, and for particle sizes of less than 0.10 micron to sizes larger than 2 microns. The function is presumably valid, however, only for systems where the particle shape approximates that of cubes or spheres—i.e., the function probably does not hold for plates or rods.

GRAPHICAL ANALYSIS

The radius value corresponding to the maximum specific extinction can be varied by changing M. Experimentally, M is varied by changing the wave length of radiation. The particle size method depends on the fact that each wave length emphasizes a different part of the particle size distribution. In order to make rapid analyses possible a graphical comparison meth has been adopted which involves the use of the two-paramet distribution function shown in Figure 3 and first used Lansing and Kraemer for molecular weight analysis (*4*).

In the analytical expression r_0 is the radius value of t maximum of the distribution and B is a nonuniformi coefficient. A B value of 0 corresponds to a sample which completely homogeneous with respect to particle size. As increases, the spread of the curve becomes greater and a value of unity corresponds to a very polydisperse materi Figure 3 shows graphically the way in which the distributi is affected as the B value is changed. As Lansing has show any of the usual averages can be expressed in terms of the t parameters r_0 and B. The expression which modifies the ponential term is derived so that the area under the dist bution curve will be unity for all values of the paramete The weight average radius, $\bar{r}$, which is usually used to ch acterize the distribution, is calculated by multiplying r_0 $e^{3B^2/4}$.

The average specific extinction, $\bar{E}_M$, for a polydisperse sa ple is calculated by multiplying the ordinates of the different particle size distribution by the corresponding ordinates of t appropriate specific extinction–radius relation. If the ar under the distribution is unity before the multiplication, t area under the product curve is numerically equal to $\bar{E}$ The specific extinction–radius relation is derived from Figur by substituting the numerical value of M in the abscissa fu tion and dividing the ordinates of the solid curve by the sulting values of particle radius.

It can be shown by a consideration of the integral implied the argument above that when the values of $\bar{E}_M$ which result fr the use of the distribution function of Figure 3 are divided by and plotted against M on double logarithmic paper, a curve wi a single maximum is obtained whose shape is independent of t r_0 value used. Changes in r_0 in this type of plot merely transl the curve parallel to the M axis. Changes in B on the other ha do change the shape of the curve. As B increases (uniformity creases) the curve becomes flatter and the change in shape is companied by a vertical and horizontal displacement of the m ima of the curves. Since the shape of the curve depends only

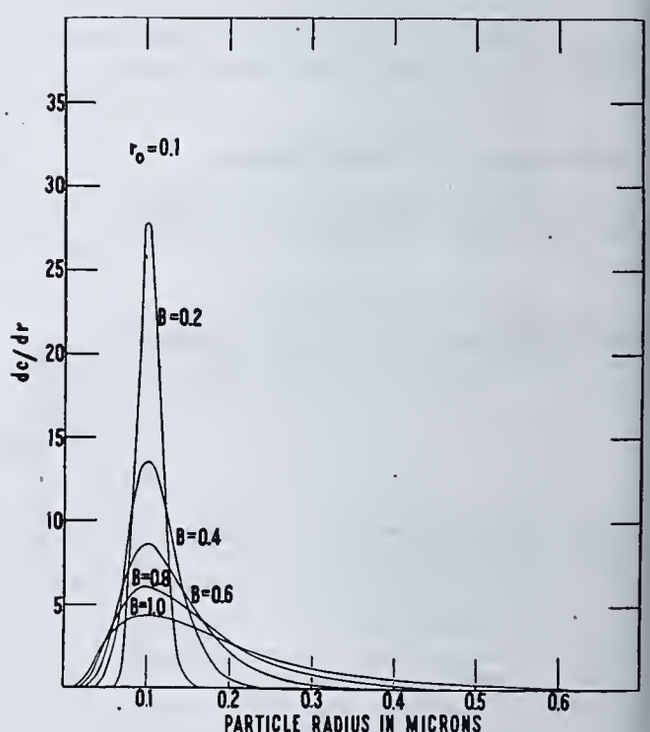

Figure 3. Distribution Function

$$\frac{dc}{dr} = \frac{e^{-\left[\frac{1}{B}\ln\frac{r}{r_0}\right]^2}}{\sqrt{\pi}\, r_0\, Be^{0.25B^2}}$$

e nonuniformity coefficient, B, this parameter an experimental distribution can be deter-ned by superimposing the experimentally termined $\frac{\bar{E}_M}{M}$ *vs.* M curve on a curve calcu-ed for the same degree of nonuniformity. It easy to show that the ratio of the M values the maxima of two curves calculated for dif-ent values of r_0 but the same value of B is ual to the ratio of the r_0 values. The r_0 value an experimental curve can therefore be deter-ned by the magnitude of the translation on logarithmic scale which is required to ng it into coincidence with a curve calcu-ed for an r_0 value of unity and the same B ue.

In order to make the comparisons suggested ve, a chart has been constructed in which $\frac{\bar{E}_M}{M}$ has been plotted against M on double loga-mic paper from values calculated for a single ue of r_0 and a range of B values. In er to make the graphical operation as simple possible, the family curves corresponding the various B values have been plotted with a common maxi-m value. Correction scales are used to compensate for the nslations which were required to make the maxima coincide.

ble I shows the values of $\frac{\bar{E}_M}{M}$ and M which were used in con-ucting the chart which is shown in Figure 4.

In Figure 4 the solid curves near the top and slightly to the ht of center are the comparison curves. The maxima of all curves have been made to agree with the maximum calcu-ed for the curve corresponding to a B value of 0. The hori-ntal M scale is shown near the center of the figure. The r_0 le above the M scale is required because an r_0 value of 0.1 cron was used in the standard calculations in order to keep chart as small as possible.

The experimentally determined points are plotted on trans-rent tracing paper, which is placed over the coordinate system the chart, so that these points can be translated easily into coin-ence with the standard family curves after they have been tted. The tracing paper is supported by a light Lucite frame ich is provided with reference lines to orient the frame with the rt and correction scales to compensate for the translations ich were required to bring the maxima of the standard family ether. The shaded frame in the figure shows the frame in plotting position.

Table I. Numerical Values Used in Constructing Standard Comparison Curves

	$\frac{\bar{E}_M}{M}$					
M	$B = 0$	$B = 0.2$	$B = 0.4$	$B = 0.6$	$B = 0.8$	$B = 1.0$
0.5	6.0	6.4	7.2	7.5	8.8	9.3
0.6	7.1	7.5	8.3	9.0	9.8	10.1
0.7	8.2	8.6	9.4	10.2	10.7	10.3
0.8	9.3	9.7	10.5	11.2	11.2	10.4
0.9	10.5	10.8	11.6	12.0	11.7	10.4
1.0	11.6	11.9	12.5	12.5	11.8	10.4
1.1	12.7	13.0	13.4	12.8	11.8	10.3
1.2	13.7	13.9	13.9	13.0	11.6	10.1
1.3	14.6	14.7	14.2	12.9	11.4	9.9
1.4	15.5	15.3	14.4	12.8	11.1	...
1.5	16.2	15.7	14.4	12.6	10.85	...
1.6	16.7	15.9	14.1	12.2	10.5	...
1.7	16.8	15.7	13.8	11.8	10.1	...
1.8	16.6	15.2	13.4	11.5	9.8	...
1.9	15.7	14.5	12.9	11.0	9.4	...
2.0	14.6	13.8	12.4	10.6	...	...
2.1	13.5	13.0	11.8	10.2	...	...
2.2	12.6	12.2	11.1	9.8	...	...
2.3	11.7	11.4	10.6	9.3	...	...
2.5	10.1	9.9	9.6	8.5	...	...
2.8	8.3	8.2	8.3	7.5	...	...

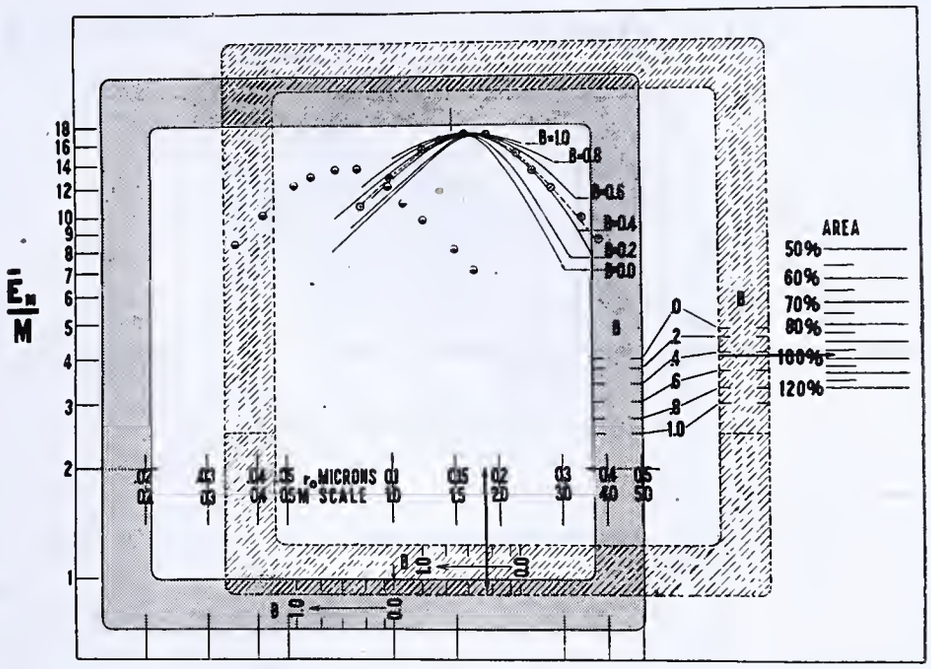

Figure 4. Standard Chart

To orient the frame for plotting, the horizontal reference lines are placed in coincidence with an $\frac{\bar{E}_M}{M}$ value of 2.0 and the vertical reference lines are adjusted to agree with an M value of 1.0. The half-shaded points shown were determined for a sample of the anatase form of titanium dioxide in a suspension medium of an alkyd varnish. These points were plotted with frame in the position indicated by the shaded rectangle. After the points have been plotted the frame is translated vertically and horizontally until the maximum of the experimental curve agrees with the maximum of the standard family, always keeping the edges of the frame parallel with the coordinate system of the chart. The translated position of the frame is shown by the cross-hatched rectangle in Figure 4. The position of the experimental points in the standard family is indicated by the open points.

The B value is determined by inspection and the r_0 value is read on the r_0 scale opposite the selected B value on the horizontal correction scale on the frame. The value read on the vertical scale to the right opposite the selected B value on the vertical correction scale shows the fraction of the material in the suspension which contributed appreciably to the scattering. When a sample contains a fraction of very small or very large material, the vertical scale readings are less than 100%. When the form of the true distribution of the sample is appreciably different from the function shown in Figure 3, the shape of the experimental curve differs from that of the standard family, and when the true distribution is more symmetrical than the distribution function the vertical scale readings are usually greater than 100%. This results from the fact that in a more symmetrical distribution there is more than the calculated amount of material in the most effective size range. In such cases the parameters are determined from the arm of the experimental curve which agrees better with the standard family.

When B has a value of 0.5 the range of r_0 which can be determined by the method varies from 0.08 to 0.5 micron for a material with a refractive index of 2.7 to 0.10 to 0.8 micron for a material with a refractive index of 2.0.

MEASUREMENT OF TRANSMISSIONS

The significance of the measurement is entirely dependent on the degree of deflocculation attained in the suspensions prepared. The suspension media are chosen to promote good dispersion and, in addition, if required, special defloculating agents are added. The suspension media are always liquids of high viscosity to retard settling. Water-white glycerol and certain alkyd varnishes give excellent results with many materials.

The suspensions usually are prepared with a carefully ground mortar and pestle. Weighed amounts (0.01 to 0.05 gram) of the material to be suspended are transferred to the mortar and the medium is added drop by drop at start. The degree of deflocu-

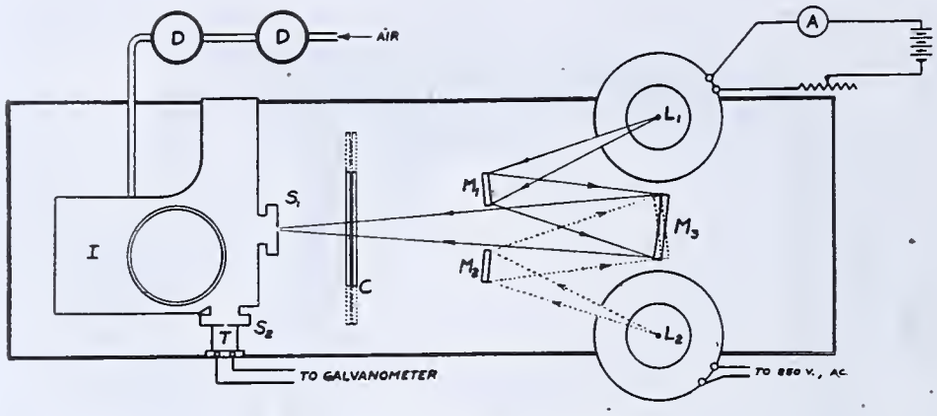

Figure 5. Experimental Arrangement

Table II. Spectral Transmission Data

Cell thickness = 0.08 cm.
Concentration = 1.19×10^{-4} cc./cc.
$ct = 0.0955$ ($c_0t_0 = 10^{-4}$)

$$\bar{E}_M = \frac{1}{ct} \times \ln \frac{1}{T} = 10.47 \times \ln \frac{1}{T}$$

Wave length, Micron	$\frac{1}{T}$	$\ln \frac{1}{T}$	$\bar{E}_M$	M	$\frac{\bar{E}_M}{M}$
0.405	3.19	1.16	12.15	1.71	7.11
0.436	3.24	1.18	12.32	1.51	8.19
0.546	2.30	1.16	12.17	1.10	11.16
0.500	3.16	1.16	12.07	1.23	9.81

lation attained depends on the amount of working that is given the paste with pestle as the first drops are added. When 1 cc. of suspension medium has been added the dilution is carried on more rapidly until the required weight of liquid has been added. The concentration is adjusted so that transmission in the blue part of the spectrum has a value of about 20%. The suspensions used in determining the distributions shown below were prepared following a time schedule in which the total time required was 0.5 hour.

Careful measurements have shown that the particle size distribution does not change with dispersion time when the time of preparation is longer than 0.5 hour. This indicates that no true grinding is done and that the degree of deflocculation is satisfactory.

A very thin cell of accurately known thickness is used in the measurements because most suspension media absorb at wave lengths between 1.5 and 2.0 microns and the effect of this absorption can be minimized by increasing the suspension concentration and reducing the cell thickness. The cell thickness now used for most of the measurements is about 0.075 cm.

The data required for the analysis are a series of 14 transmissions measured over the wave-length range of 0.4 to 2.0 microns. Figure 5 shows the experimental arrangement which has been used for such measurements.

L_1 is an incandescent lamp and L_2 is an H-3 mercury arc. M_1 and M_2 are plane mirrors which permit interchanging the sources and M_3 is a concave mirror which produces an image of the source at the monochromator slit, S_1. The monochromator, I, is based on a Gaertner Type L 235 infrared spectrometer which was modified by manufacturer to cover the full spectral range given above. T, the receiver, may be either a vacuum thermopile or a photocell. According to the present operating practice a photocell is used with an amplifier and meter for the visible spectrum and a thermopile-galvanometer combination used for the spectral region from 0.7 to 2.0 microns. The mercury arc is used in the blue and violet parts of the spectrum. The incandescent lamp is used for wave lengths of 0.5 micron and longer. The cell block containing three cells, two for suspensions and one for the clear suspension medium, is shown at C.

The specific extinctions are calculated from the measured transmissions in the manner indicated in Table II.

The factor which converts the logarithm reciprocal transmission to specific extinction calculated from the values of concentration a cell thickness as indicated at the top of t table. Column 2 shows the reciprocal of t transmissions measured at the wave lengths column 1. Columns 3 and 4 show the calcul tion of specific extinction. The M values column 5 are calculated for anatase in an alk varnish and 6 is obtained by dividing 4 by Columns 5 and 6 are plotted as shown Figure 4.

The method of analysis is rather rapid, t complete operation requiring but 2 hours, a the operations involved are simple enough for technician to carry out satisfactorily. The r sults are usually reported as average radius, the B value foun and the fraction of the material found in the distribution.

COMPARISONS WITH ULTRACENTRIFUGE DATA

The spectral transmission method of particle size analysis h been in use for several years and during that time its reliabili has been reasonably well tested. Figures 6 and 7 show the w in which the method has operated with practical systems.

Figure 6 illustrates the effectiveness of B as a measure of no uniformity.

Distributions calculated from the values of r_0 and B are co pared with distributions determined with the ultracentrifuge f the same materials. In both samples of anatase shown the val of r_0 is about 0.2 micron. Anatase A has a B value of 0.2 as result of careful laboratory fractionation, while anatase B has B value of 0.6 and is comparable with the material which w fractionated to obtain distribution A. The distribution for t bentonite clay has a B value of 0.95. The agreement obtain indicates that B is a satisfactory measure of uniformity.

The anatase form of titanium dioxide has a refractive index 2.5 at the D line, while bentonite has a refractive index of abo 1.5 and was measured in a medium whose refractive index w 1.4 Figure 7 shows a continuation of distribution compariso for materials of different refractive index. The section at t left shows a sample of the rutile form titanium dioxide, the r fractive index of which is about 2.7 at the D line. The center se tion shows an experimental sample, the refractive index of whi

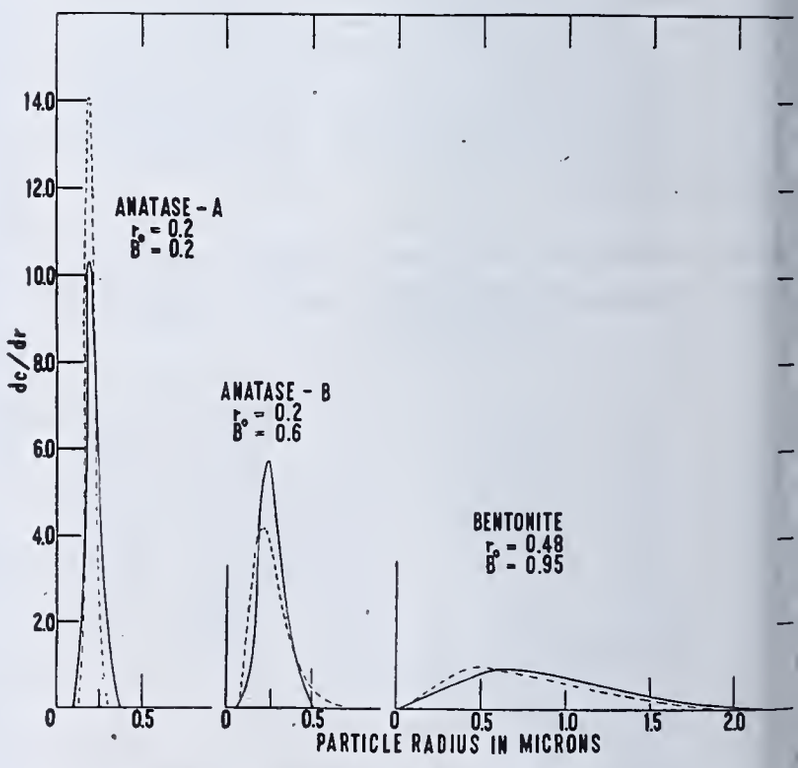

Figure 6. Comparison of Particle Size Distributions

——— Ultracentrifuge
- - - - - Spectral transmission

was found to be 2.25 at the D line, and the distributions shown were used in determining this refractive index. The section at the right shows a comparison for a sample of zinc oxide, the relative refractive index of which is about 2.0 at the D line. The spectral transmission distribution for the zinc oxide sample was measured in a solid medium. The sample was prepared by dispersing the pigment in partially polymerized methyl methacrylate resin and the distribution determined when the polymerization was complete. This example illustrates the fact that the method is not limited to liquid media.

The method can be useful with unstable systems. Measurements for such systems have been made with a recording spectrophotometer similar to the instrument now made by the General Electric Company. The time required for the measurement of the full spectral range curve of the instrument is only 3 minutes and this speed of measurement makes such analyses possible. The restricted wave-length range of this spectrophotometer, however, makes it advisable to use the more cumbersome apparatus described earlier for routine measurements.

While different operators will choose values of B which will vary by 0.1, or in some cases more, the corresponding differences in r_0 usually compensate. The work which has been carried out indicates that the average radius is reproducible to within 5%.

REFRACTIVE INDEX

It has previously been assumed that the M value for each wave length of radiation in the range of measurement was known. In order to calculate such M values it is necessary to know the refractive index of both particle and suspension medium over the wave length range of 0.4 to 2.0 microns. The refractive indices of most materials of interest are given in literature for the visible part of the spectrum. In the work reported, the values for the infrared have been calculated by the simple Hartmann dispersion formula $n = n_0 + \frac{c}{\lambda - \lambda_0}$ where n_0, c, and λ_0 are arbitrary constants.

In Figure 8 the indices of several materials are shown to illustrate the use of this method, and the corresponding M values are plotted against wave length below. Satisfactory results have been obtained by using the average index of anisotropic materials, so long as the particle index is not close to that of the suspension medium. When one index of a material with multiple indices nearly matches the medium, the method does not give reliable results.

When no refractive index data are available for materials whose refractive index is above 2.0, there has been no very reliable method for determining the index if the material is available only in finely divided form. A method for making such determinations has been worked out which makes use of the graphical technique described.

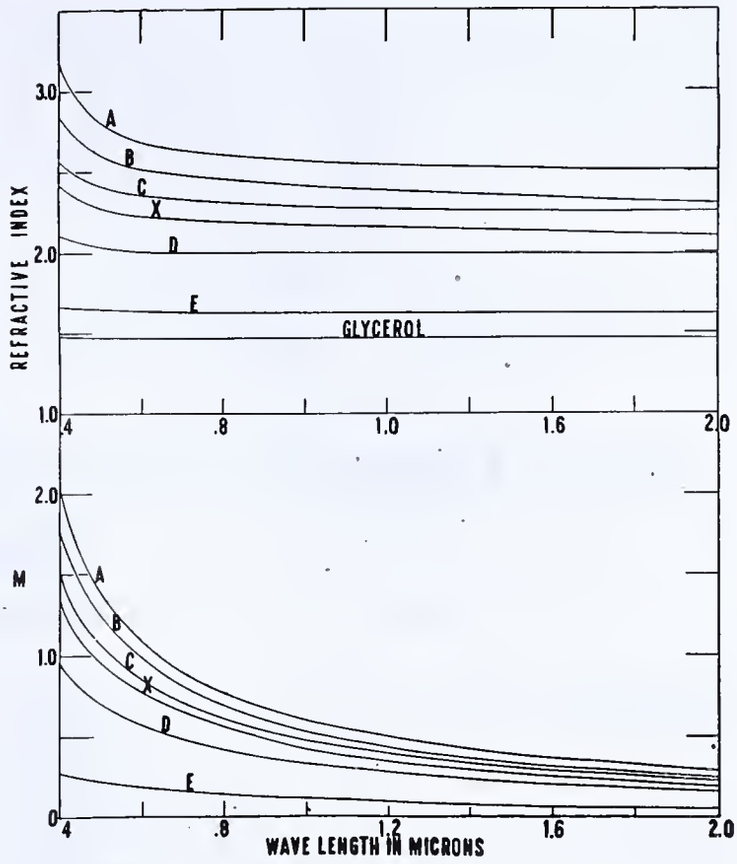

Figure 8. Refractive Index and M *vs.* Wave Length

A. Rutile, TiO_2 *D.* Cassiterite, SnO_2
B. Anatase, TiO_2 *E.* Barytes, $BaSO_4$
C. Sphalerite, ZnS *X.* Experimental sample

In order to make this evaluation it is necessary to determine the particle size distribution of the material by another method, such as ultracentrifugal analysis. An approximate value of M is then calculated from the average radius and a measured specific extinction. Using this value an M *vs.* λ curve is interpolated in the series of curves of Figure 8 and the graphical method applied with the measured specific extinction *vs.* wave length curve. The distribution calculated with the trial values of M usually is in error but it does provide a basis for selecting a better value of M to use in a second attempt. By continuing this process of successive approximations, it is possible to calculate a reasonably accurate curve showing M as function of wave length. When the vertical scale reading in Figure 4 is very nearly 100% for an M *vs.* λ curve which gives parameters which agree with the known distribution, it is fairly certain that the dispersion deduced is accurate. The refractive indices can, of course, be calculated from the M values.

The curves marked X in Figure 8 were determined in this way. The agreement obtained between the ultracentrifuge distribution and the distribution calculated by the graphical analysis using the final M values is shown in the middle section of Figure 7.

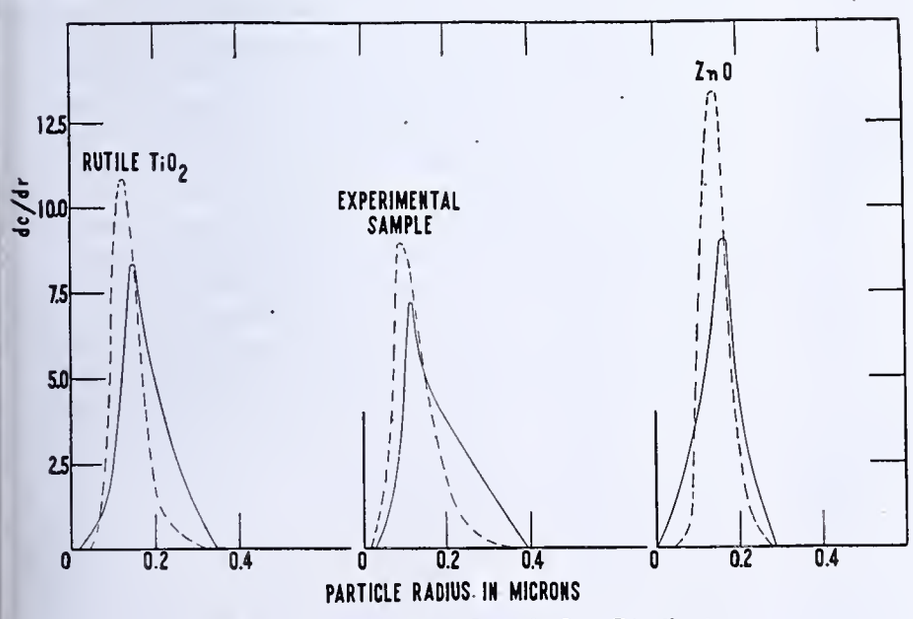

Figure 7. Comparison of Particle Size Distributions

———Ultracentrifuge
– – – –Spectral transmission

ACKNOWLEDGMENTS

Grateful acknowledgment is made of the important contribution which Elmer O. Kraemer made to this development. All the work on scattering systems referred to here was carried out in the research group which he headed and much of what was accomplished was due to his active participation.

The work on scattering systems was carried out in collaboration with J. Burton Nichols, who supplied many helpful comments on the present development. Many of the measurements reported were carried out by O. H. Graeger, who also worked out the details of the method for determining refractive index.

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Rapid Method for Determining Specific Surface of Fine Particles

ALPHONSE PECHUKAS AND F. W. GAGE, Pittsburgh Plate Glass Co., Columbia Chemical Division, Barberton, Ohio

The Lea and Nurse method of determining the specific surface of fine powders using gas permeabilities has been modified for measuring particles whose mean diameters are less than 1.0 micron. The problem of turbulence within the porous sample plug which accompanies measurements of finely divided pigments has been overcome by using small-bore, highly compressed plugs. Pressure differentials of 1 atmosphere have been substituted for the lower pressures of Lea and Nurse to overcome the resistance to air flow offered by the more highly compressed plugs. The method is rapid and simple in operation and is reproducible, the specific surface values being fairly constant over a range of porosities. An apparatus suitable for routine work and plant control is described.

DURING the past several years the permeability method of determining the specific surface of fine particles has received considerable attention from workers interested in a rapid and accurate means of measuring the particle size of pigments in the subsieve range. The theory of the permeability method and details of experiments conducted to test its validity have been discussed by Carman (*4–6*), who showed that the specific surface of a powder could be expressed by the equation

$$S_0 = 14 \sqrt{\frac{1}{K_1} \times \frac{\epsilon^3}{(1 - \epsilon)^2}} \qquad (1)$$

where

S_0 = specific surface of the powder in sq. cm. per cc.
K_1 = proportionality constant representing the permeability of the porous medium
ϵ = porosity or fractional void of the bed of powder

Based on laws of fluid flow, K_1 can be expressed by

$$K_1 = \frac{Q_1 \eta L}{A \times \Delta P} \qquad (2)$$

where

Q_1 = rate of flow of the percolating fluid in ml. per second
A = cross-sectional area of the porous medium in sq. cm.
η = viscosity of the fluid in poises
L = thickness of porous medium in cm.
ΔP = pressure difference driving the fluid through the medium in grams per sq. cm.

Carman has shown that for the same powder at different porosities the porosity function, $\epsilon^3/(1 - \epsilon)^2$, is accurate over a fairly wide range of porosities.

Carman worked for the most part with liquids rather than air, being of the opinion that surface area values from air permeabilities tended to be high, owing to incomplete dispersion of the powder in the bed. However, he recognized that not only was it difficult to select a liquid that would give reasonably good dispersion and wetting, but adsorption of the liquid at the particle surface might tend to decrease the effective porosity, giving a low permeability and therefore a high surface area value.

Lea and Nurse (*9*) have evolved a highly developed gas-permeability method which is appreciably more rapid than the liquid-permeability method and is extremely reproducible. They have shown that gas permeabilities are consistent for different gases and that corresponding values of particle size with gases are more in accord with sedimentation particle size analysis than with liquids.

Gooden and Smith (*7*) have also successfully used air permeabilities for measuring various sized fractions of powdered silica. However, for the more finely divided fractions, agreement was poor between the air permeability particle size and that calculated from microscopic measurements.

Blaine (*2*) has devised an air-permeability apparatus similar to that of Lea and Nurse which he found well adapted to comparing the specific surfaces of powdered materials. His procedure was fast and simple and the test results were reproducible. He concluded that the air-permeability apparatus was not only well adapted for testing materials in the range of fineness of portland cement but could be used for testing finer materials.

In a more recent article Blaine (*3*) describes a simplified air-permeability apparatus suitable for rapid comparison of the specific surfaces of portland cements. Not only is the apparatus simple to construct and operate, but times of less than 3 minutes are required for passing air through the sample bed.

Under the auspices of the A.S.T.M. (*1*) a comparative test of the Lea and Nurse air-permeation method was conducted on eight portland cements. Twenty laboratories participated using twenty different instruments. The agreement between laboratories was reasonably good, especially when the cements were tested at average porosities. However, there appeared to be a regular increase in specific surface values with decrease in porosity. It was proposed that a change in the Lea and Nurse formula be made by which the values obtained for specific surface would be more nearly the same regardless of the porosity at which determined. This change took the form of a constant which could be determined by plotting a function of the surface areas as determined at different porosities against the porosities at which they were determined and extrapolating the straight line obtained to zero surface area. The intersection at the zero surface area line is apparently a constant for each type of material. This constant

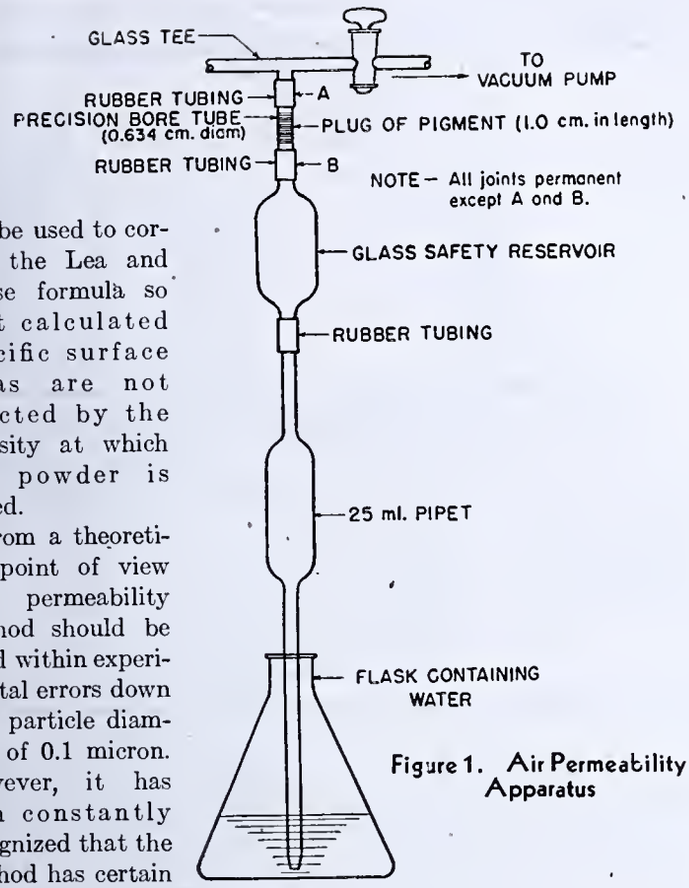


Figure 1. Air Permeability Apparatus

can be used to correct the Lea and Nurse formula so that calculated specific surface areas are not affected by the porosity at which the powder is tested.

From a theoretical point of view the permeability method should be valid within experimental errors down to a particle diameter of 0.1 micron. However, it has been constantly recognized that the method has certain limitations which might not permit its being applied to the measurement of particle size diameters much below 2 microns. Lea and Nurse (*9*) have stated that the method may be limited to particles of 10 microns or larger. Work (*10*) has found that the porosity function, $\epsilon^3/(1-\epsilon)^2$, is very critical and is not proportional to the permeability over a very wide range in some cases. Thus in making tests of various materials it is necessary to determine the porosity at which this function is proportional to the permeability.

However, because of its relative simplicity and apparent reproducibility the method definitely merits consideration.

A need arose in this laboratory for a rapid, reproducible method for determining the relative particle sizes of pigments in the range of 0.1 to 2.0 microns. Microscopic methods were tedious and only semiquantitative, since with even a dark-field microscope the smaller particles are often overlooked and a true evaluation cannot be made. Adsorption methods are also time-consuming and often lead to erroneous results, especially with particles of irregular surfaces. Attention was therefore turned to an air-permeation method. An investigation of the method detailed by Lea and Nurse (*9*) demonstrated that some modifications would have to be made before it could be used satisfactorily to measure the specific surface of pigments having mean diameters less than 1.0 micron. Air flow rates much in excess of 10 ml. per minute were to be avoided since turbulence resulted, invalidating the equation developed by Carman (*4*) for Poiseuille flow. It was also found necessary to compact the pigment tightly in small-bore tubes in order to avoid channeling, which resulted if only relatively light pressures and large beds were used. These highly compressed pigment beds resisted air flow to such an extent that measured rates could be obtained only by using a full atmosphere pressure drop across the bed.

APPARATUS

The essential features of the apparatus first developed for determining the surface area of finely divided pigments are shown in Figure 1.

It consists of an Erlenmeyer flask filled with water, a 25-ml. pipet connected to a glass safety reservoir with a permanent heavy rubber connection, and the sample tube, having an internal diameter of 0.634 cm. and constructed of precision-bore tubing.

In making a determination a plug of pigment of known volume is formed in the sample tube. This is packed in by hand, using two short cylindrical lengths of metal which fit snugly inside the sample tube and extend beyond it at each end. The length of the plug is measured with a micrometer which spans the plug and the two metal pieces of known length. To simplify calculations the pigment plugs are all formed to a constant length of 1.00 cm. This is done by adding or subtracting pigment until the well packed plug is slightly too long and then making a final adjustment by compressing to the exact length.

The sample tube is then weighed and placed in the apparatus. The flask of water is drawn to one side, so the pipet tip is no longer immersed in the water, and the vacuum pump started. The stopcock on the vacuum line is then opened slowly to avoid sudden application of vacuum to the sample plug. The pressure differential is noted by means of a mercury gage (not shown in Figure 1) and maintained at approximately 740 mm. of mercury. After permitting the system to come to equilibrium, the rate of flow of air through the sample plug is measured by moving the flask of water back to position and timing accurately the interval required to draw 25 ml. of water into the pipet.

With the data obtained it was possible to calculate the specific surface of the sample. Equations 1 and 2 may be combined and modified to yield the following equation:

$$S_0 = 14\sqrt{\frac{At \times \Delta P}{Q_2 \eta L} \times \frac{\epsilon^3}{(1-\epsilon)^2}} \qquad (3)$$

where

Q_2 = volume of air in ml. flowing through sample plug in time t

or

$$S_w = \frac{S_0}{\rho} = \frac{14}{\rho}\sqrt{\frac{At \times \Delta P}{Q_2 \eta L} \times \frac{\epsilon^3}{(1-\epsilon)^2}} \qquad (4)$$

where

S_w = specific surface of the pigment in sq. cm. per gram
ρ = specific gravity of pigment

Since ρ, A, ΔP, η, and L are known constants, Equation 4 may be put in the following form:

$$S_w = K_2\sqrt{\frac{t}{Q_2} \times \frac{\epsilon^3}{(1-\epsilon)^2}} \qquad (5)$$

where

$$K_2 = \frac{14}{\rho}\sqrt{\frac{A \times \Delta P}{\eta L}}$$

K_2 will, of course, have a unique value for each type of pigment. The porosity may be expressed mathematically as

$$\epsilon = 1 - \frac{W}{\rho A L} \qquad (6)$$

where

W = weight of pigment in plug in grams

Having measured t, Q_2, and W, it is a very simple task to combine Equations 5 and 6 to calculate the specific surface, S_w

It is clear from the foregoing that the apparatus required and technique are very simple. The method is also rapid, requiring less than an hour for one determination. If it is desired, more than one apparatus may be run off the same vacuum manifold.

The reproducibility is good, as is indicated in Table I. Small variations in ΔP which are experienced because of fluctuations in the barometric pressure have little effect on the specific surface values. Although no attempt is made to use the same weight of pigment for each determination, the porosities are fairly uniform in duplicate determinations with the same pigment.

Because of the critical nature of the porosity function, $\epsilon^3/(1-\epsilon)^2$, the actual values of surface area may not agree too well with those obtained by this method. However, since no known method can be relied upon to yield infallible surface area measurements, comparisons with other methods, while not precise, serve to indicate whether the method yields results of the right order of magnitude. Harkins and Gans (*8*) using an adsorption method obtained values of 38,000 and 57,300 sq. cm. per gram on two titanium dioxide pigments, which compare very favorably with the results in Table I. Apparently the air-permeation method described here can be relied upon to give values of the correct order of magnitude.

Table I. Surface Area of Titanium Dioxide Pigment

Pigment	Porosity	Surface Area, Sq. Cm. per Gram
Titanium dioxide 1	0.7194	36,600
	0.7337	36,200
	0.7286	35,400
	0.7167	34,900

Table II. Comparison of Mean Cross-Sectional Diameter Values

(Calculated from specific surface value of a titanium dioxide pigment, assuming various particle shapes. Specific surface area, 36,000 sq. cm. per gram)

Assumed Shaped of Particles	Mean Diameter, Micron
Spherical	0.42
Rod-shaped, length 1.5 times diameter	0.37
Rod-shaped, length 2 times diameter	0.35
Rod-shaped, length 3 times diameter	0.33
Rod-shaped, length 4 times diameter	0.31

APPARATUS FOR ROUTINE CONTROL WORK

Although this method was highly satisfactory as a research tool, it soon became desirable to modify it for routine and more rapid determinations. Therefore, the apparatus was redesigned so that shorter times would be required for making a determination, and calculation of the particle size would be simplified.

This modified apparatus, diagrammed in Figure 2, consists of a 150-ml. beaker filled with water, an inverted 10-ml. graduated pipet with the tip cut off, a calcium chloride tube, the sample tube, and a stopcock, which is connected to a source of high vacuum. The sample tube is a stainless steel tube carefully machined and having an internal diameter of 0.579 ± 0.0025 cm. All connections are made with heavy, tightly fitting sections of rubber tubing and are all made permanent except those between the calcium chloride tube and the rubber stopper and between the sample tube and the rubber connection.

In making a determination the sample tube is weighed and then placed over the bottom plunger of the hand press shown in Figure 3. The sample is transferred to the sample tube by means of a funnel and packed into the tube, using the top section of the hand press. When the plug is reasonably firm the sample tube is reversed. This prevents the tube from coming in contact with either the top or bottom of the hand press, thus ensuring that the plug length is exactly that between the two plungers. Sample is added until a firm, solid plug is obtained. Any portion of the sample which might adhere to the sides of the tube above the surface of the plug is loosened with a sharp-pointed wire and removed. The tube is again weighed and placed in the apparatus, the two nonpermanent connections being made air-tight. The stopcock is then cautiously opened to the vacuum, held at less than 5-mm. pressure. About one minute is allowed for the system to come to equilibrium, after which the time for a measured amount of air to pass through the plug is determined. The quantity of air is chosen so that the time required is between 2 and 5 minutes.

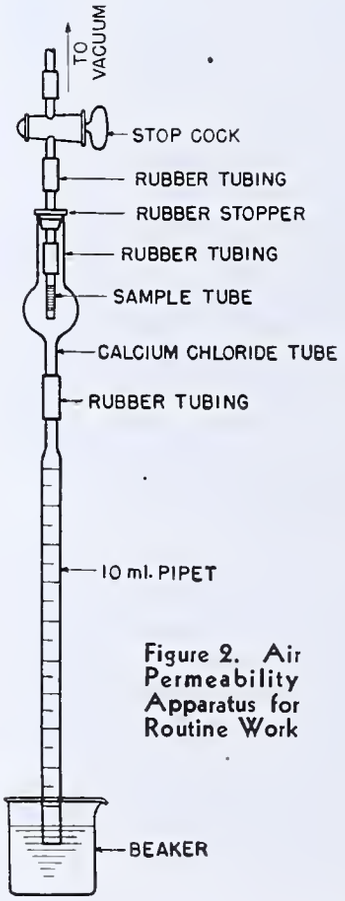


Figure 2. Air Permeability Apparatus for Routine Work

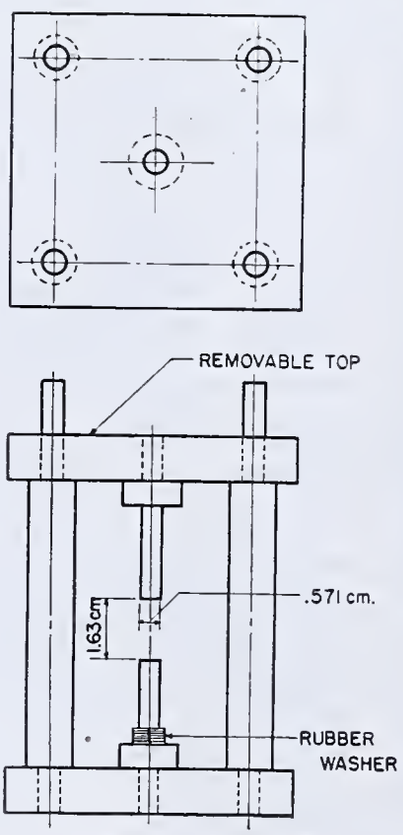


Figure 3. Hand Press

Table III. Mean Particle Diameters of Pigments, Using Rapid Routine Method

Pigment	Weight of Plug *Gram*	Specific Gravity of Pigment *Gram/ml.*	Volume of Air *Ml.*	Time *Sec.*	d_m *Micron*
Titanium dioxide 2	0.6963	4.00	5.0	151	0.74
	0.7376		5.0	169	0.78
	0.6623		7.0	161	0.78
	0.6624		5.0	116	0.78
Calcium carbonate 1	0.3424	2.70	3.0	181	0.30
	0.3177		3.0	156	0.28
	0.3394		3.0	195	0.28
Calcium carbonate 2	0.4432	2.70	2.5	177	0.43
	0.4313		2.5	186	9.40
	0.4225		3.0	218	0.38
	0.4346		3.0	224	0.40

Table IV. Possible Errors in Mean Diameter Determination

Variable	Accuracy Assumed in Measuring Variable	Maximum % Deviation in d_m Due to Error in Measuring Variable
Volume of air, Q_2	±0.1 ml.	±1.0
ΔP	±25 mm. of mercury	±1.7
Time, t	±1 sec.	±0.4
Length of plug, L	±0.01 cm.	±0.3
Area of plug, A	$\pm 2.3 \times 10^{-3}$ sq. cm.	±0.5
Porosity of plug, ϵ	$\pm 2.3 \times 10^{-3}$ sq. cm. (A)	±2.0
	±0.01 cm. (L)	±2.0
	±0.001 gram (W)	±0.5
	±0.02 gram per ml. (ρ)	±1.2

CALCULATION OF PARTICLE SIZE

For routine control it is convenient to express the specific surface area of the pigment in terms of mean diameter. This is usually done by assuming a spherical shape for each particle. However, whether a spherical shape or a rodlike shape is assumed does not change the order of magnitude of the mean cross-sectional diameter, and in fact does not change the actual value appreciably. This is demonstrated in Table II, in which the mean cross-sectional diameters of a titanium dioxide pigment calculated on the basis of several assumed particle shapes are presented.

Assuming a spherical particle, Equation 4 becomes

$$S_w = \frac{6}{d_m \rho} \tag{7}$$

where

d_m = mean cross-sectional diameter in cm.

By combining Equations 4 and 7 and expressing d_m in microns, the following equation is obtained:

$$d_m = \frac{30{,}000}{7} \times \sqrt{\frac{Q_2 \eta L (1-\epsilon)^2}{\epsilon^3 A t \Delta P}} \tag{8}$$

By suitable mathematical expansion Equation 8 becomes

$$d_m = \left[\left(\frac{3 \times 10^4}{7} \times L \sqrt{\frac{\eta}{\Delta P}}\right)\left(\frac{W/\rho}{(LA - W/\rho)^{3/2}}\right)\right]\sqrt{\frac{Q_2}{t}} \tag{9}$$

$$= K_3 \sqrt{\frac{Q_2}{t}}$$

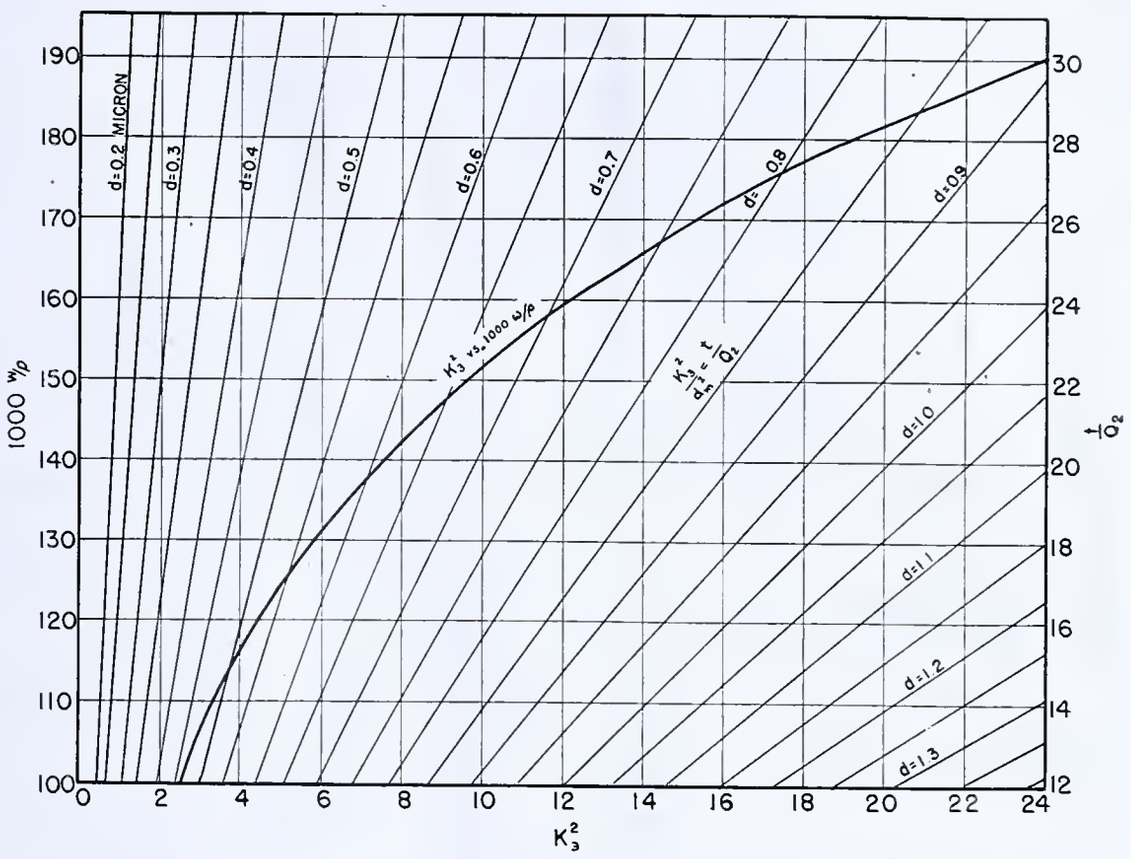
where

$$K_3 = \left(\frac{3 \times 10^4}{7} \times L \sqrt{\frac{\eta}{\Delta P}}\right)\left(\frac{W/\rho}{(LA - W/\rho)^{3/2}}\right) \tag{9A}$$

or

$$\frac{K_3^2}{d_m^2} = \frac{t}{Q_2} \tag{10}$$

By employing Equations 9A and 10 the value of d_m may be calculated by a simple graphical method. How this can be accomplished is shown in Figure 4.



gure 4. Chart for Determining lean Cross-Sectional Diameter

Since L, η, and A are constants and ΔP is held within nits, variations within which ve only negligible effect on e value of d_m, the value of , or K_3^2, is a function of the tio of the weight, W, of the mple to its specific gravity, and a curve is drawn relating ese two functions. On the me graph is drawn a family curves in which K_3^2 is plotted rsus t/Q_2 for a series of d_m lues covering those of the gments being measured. A y preliminary measurements ll determine what range of Q_2 and W/ρ values will be countered and therefore what nges must be covered by the aph. To determine the value d_m for any particular pigent, ratios W/ρ and t/Q_2 are culated. Using the W/ρ sus K_3^2 curve, the K_3^2 value responding to the W/ρ value determined. The intersecn of this K_3^2 value with the Q_2 ratio is then found and the ue of d_m obtained by interation.

This method is reproducible, as is demonstrated in Table III. was recognized that there would be several sources of error in measurement of this type. Reference to Table IV will demtrate, however, that if all errors were cumulative the maxim deviation in the value of d_m would be 9.6%, owing to experintal errors. In actual practice the maximum deviation is approximately 5% of the value of d_m. This does not appear too ge in light of the errors accompanying measurements of mean ss-sectional diameters by other methods.

SUMMARY

A method for determining the specific surface area of pigments the range of 0.1 to 1.0 micron has been described. The method reproducible and is easily modified for rapid control work. Although the specific surface values obtained may not be true val, accurate comparisons of similar pigment types may be made.

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Presented before the Division of Industrial and Engineering Chemistry, American Chemical Society, Symposium on Measurement and Creation of Particle Size, Brooklyn, N. Y., December, 1945.

Simultaneous Determination of Hydrogen Sulfide and Carbon Dioxide in a Continuous Gas Stream

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A method is described for the simultaneous determination of hydrogen sulfide and carbon dioxide in a continuous stream of natural gas. The two acidic gases are absorbed in standardized cadmium and barium hydroxide solutions. Only simple laboratory equipment is required.

THE course of one of the research projects of this laboratory, it was found necessary to analyze a stream of natural gas for hydrogen sulfide and carbon dioxide in such a manner that a large number of analyses could be taken which, at the end of the operation, could be integrated into a quantitative over-all balance.

Various methods are known for determining hydrogen sulfide and carbon dioxide in natural gas. Hydrogen sulfide is most commonly determined by the Tutweiler method (*2*, *4*, *9*), or by one of the cadmium sulfide methods (*3*, *5*). The carbon dioxide content of the gas is usually measured by absorption in sodium hydroxide solution in an Orsat (*2*, *6*, *8*) or Orsat-type apparatus, or may be determined by absorption in barium hydroxide solution as described by Martin and Green (*7*). When both hydrogen sulfide and carbon dioxide are present, the total acid gas content

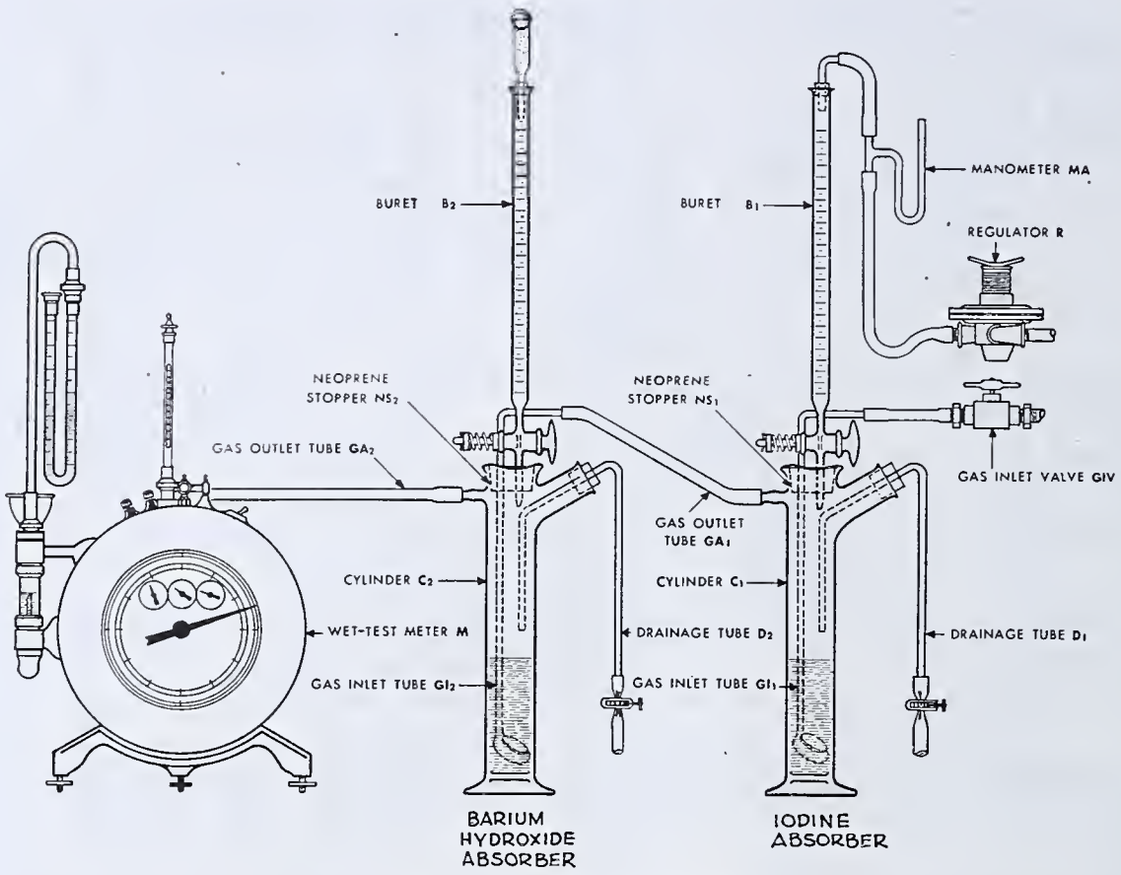

Figure 1. Diagram of Apparatus

is measured by absorption in sodium hydroxide, the hydrogen sulfide determined by one of the methods mentioned, and the carbon dioxide found by difference.

These methods were not satisfactory for the project under investigation, mainly because they did not provide means for analyzing the gas stream continuously and simultaneously for both hydrogen sulfide and carbon dioxide. Results obtained by the Orsat and Tutweiler methods represent merely individual spot analyses, and do not indicate gradually occurring changes of the hydrogen sulfide and carbon dioxide content. For the same reason, the over-all balance which is calculated from such analyses is inaccurate. By using the cadmium sulfide method, the gas stream can be analyzed continuously for hydrogen sulfide, but the carbon dioxide content still has to be found by spot analyses. Furthermore, the equipment specified by the C.N.G.A. (*5*) for this method is complicated and difficult to clean, which causes some difficulties in an operation that requires many analyses.

The method which this laboratory has developed consists of titrating the hydrogen sulfide with standard iodine and the carbon dioxide with standard barium hydroxide solution, using starch and phenolphthalein as the respective indicators. The chemical reactions upon which this method is based are:

$$H_2S + I_2 = 2HI + S \quad (1)$$

$$Ba(OH)_2 + CO_2 = BaCO_3 + H_2O \quad (2)$$

The advantage of this method is that any chosen volume of gas can be analyzed by appropriate variation of the increments and the strength of the titrating fluids, the gas stream can be observed at any given moment, and occurring changes detected immediately. However, the method does not distinguish between hydrogen sulfide and mercaptans or other reducing agents. If such are present, the "apparent" hydrogen sulfide content is determined. Furthermore, any acidic constituents, other than carbon dioxide, which would be absorbed by barium hydroxide are also disturbing factors.

EXPERIMENTAL PROCEDURE

Reagents. Iodine solution, 0.1 *N*, is prepared by dissolving approximately 60 grams of pure potassium iodide in 50 ml. of water, to which 13 grams of pure iodine are then added. This solution is made up to a final volume of 1 liter with distilled water.

The iodine solution is standardized with arsenic trioxide Standard Sample 83a, or subsequent samples, of the National Bureau of Standards according to the procedure indicated in the provisional certificate of analysis of Standard Sample 83a.

Barium hydroxide solution 0.25 *N*, is prepared by dissolving 79 grams of pure barium hydroxide octahydrate in 1 liter of distilled water free of carbon dioxide.

The barium hydroxide solution is standardized with 0.1 *N* hydrochloric acid, which has been standardized against 0.1 *N* sodium hydroxide. Acid potassium phthalate Standard Sample 84b, or subsequent samples, of the National Bureau of Standards is used as a primary standard for the sodium hydroxide solution. The procedure to be followed is specified in the provisional certificate of analysis of Standard Sample 84b.

Starch solution, 0.4%.

Phenolphthalein solution, 0.1%.

Apparatus. The equipment used in this method (Figure 1) consists of two 500-ml. Pyrex hydrometer cylinders, C_1 and C_2, modified by the addition of a side arm, into the top of each of which a two-holed neoprene stopper, NS_1 and NS_2, is inserted. One of the holes holds the gas inlet, GI_1 and GI_2, which is a sintered-glass dispersion tube; through the other hole a 50-ml. buret, B_1 and B_2, is inserted in such a manner that its tip reaches into the cylinder. The side arm, GA_1 and GA_2, serves as gas outlet. D_1 and D_2 are drainage tubes, through which liquid can be siphoned from the cylinder.

B_1 is filled with the standard iodine solution for the titration of the hydrogen sulfide. Inasmuch as the gas dispersion tube in the barium hydroxide absorber creates some back pressure in the iodine absorber, it is necessary to drive the iodine solution from the buret by means of air or neutral gas at a pressure of from 15 to 250 mm. of mercury which is controlled by the regulator, *R*, and the manometer, *MA*. This pressure necessitates the use of a spring-loaded stopcock on the iodine buret. B_2 is filled with standard barium hydroxide solution for the titration of the carbon dioxide. It is necessary to close the top of B_2 with a soda-lime tube to protect the barium hydroxide from contact with carbon dioxide in the air.

The connection between the gas source, the two cylinders, and the gas meter, *M*, are made by neoprene tubing. Rubber tubing absorbs small amounts of hydrogen sulfide which causes inaccuracy in the analysis and, therefore, the use of neoprene tubing and stoppers is recommended. It is desirable to use as short lengths of this tubing as convenient, using glass tubing to span any very great distances.

The gas meter, *M*, can be any suitable displacement meter graduated in 0.01 liter or 0.001 cubic foot.

Procedure. Before the test is started, C_1 and C_2 are loaded with 150 ml. of carbon dioxide–free distilled water, 5 ml. of 0.4% starch solution and sufficient standard iodine solution to produce a slight but permanent blue coloration in the solution are added to C_1, and 5 ml. of 0.1% phenolphthalein solution are placed in C_2. B_1 and B_2 are filled with the respective titration fluids.

A measured amount of iodine and barium hydroxide solution is now added to the respective cylinders and the flow is started at the gas inlet valve, *GIV*, at a rate of 1 to 2 cubic feet per hour. As soon as the iodine or barium hydroxide solution is exhausted, which is indicated by a color change of the indicator, the gas volume read on the meter is recorded and fresh titration fluid is added. This procedure is continued throughout the entire test

eriod. Whenever the amount of liquid in the cylinder becomes o large, sufficient liquid is drained through D_1 and D_2 to allow ıe operation to be continued.

By decreasing the increments of titration fluid and the rate of ıs flow, rather rapid changes in the gas composition can readily e detected. If gas of rather constant composition is analyzed rger increments should be used. Furthermore, in cases of very w or very high concentrations of hydrogen sulfide and carbon ioxide, the strength of the titration fluids may be varied. In is laboratory, experiments run with 0.01 N, 0.025 N, 0.1 N, d 0.5 N iodine, and with 0.1 N, 0.25 N, and 0.5 N barium ydroxide solutions yielded satisfactory results.

The sulfur formed in C_1 and the barium carbonate formed in C_2 ıring the analysis somewhat impair the detection of the color ıange of the indicator and tend to block the sintered-glass plate the gas dispersion tube. Both difficulties can be overcome by me experience in the operation and the choice of coarse (such as orning "C" porosity) sintered plates.

CALCULATIONS

The calculations of three typical test runs, using gas mixtures different composition and titrating fluids of different strength, e shown in Table I.

Table I. Typical Test Runs

Total Metered Flow *u. feet*	Gas Tested *Liters*	Gas per H_2S Test *Liters*	Gas per CO_2 Test *Liters*	Iodine Increment *Ml.*	H_2S Calcd. *Ml.*	H_2S Content %	$Ba(OH)_2$ Increment *Ml.*	CO_2 Calcd. *Ml.*	CO_2 Content %
Run 1, Iodine Normality 0.0984, $Ba(OH)_2$ Normality 0.4226[a]									
.089	2.533	2.384	...	25.0	29.1	1.15	...	...	...
.098	2.763	...	2.600	...	...	...	25.0	125.0	4.52
.181	2.336	...	2.198	...	...	...	25.0	125.0	5.35
.182	2.621	2.466	...	25.0	29.1	1.11	...	...	...
.263	2.307	...	2.171	...	...	...	25.0	125.0	5.42
.273	2.563	2.412	...	25.0	29.1	1.14	...	...	...
.345	2.307	...	2.171	...	...	...	25.0	125.0	5.42
.365	2.592	2.439	...	25.0	29.1	1.12	...	...	...
.425	2.251	...	2.118	...	...	...	25.0	125.0	5.55
.458	2.621	2.466	...	25.0	29.1	1.11	...	...	...
.507	2.307	...	2.171	...	...	...	25.0	125.0	5.42
.549	2.563	2.412	...	25.0	29.1	1.14	...	...	...
.588	2.278	...	2.144	...	...	...	25.0	125.0	5.49
.642	2.621	2.466	...	25.0	29.1	1.11	...	...	...
.671	2.336	...	2.198	...	...	...	25.0	125.0	5.35
.735	2.621	2.466	...	25.0	29.1	1.11	...	...	...
.753	2.307	...	2.171	...	...	...	25.0	125.0	5.42
.826	2.563	2.412	...	25.0	29.1	1.14	...	...	...
.835	2.307	...	2.171	...	...	...	25.0	125.0	5.42
.918	2.563	2.412	...	25.0	29.1	1.14	...	...	...
Run 2, Iodine Normality 0.00955, $Ba(OH)_2$ Normality 0.2306[b]									
.0085	2.302	2.244	...	25.0	2.83	0.12	...	...	...
.129	3.508	...	3.419	...	...	...	25.0	68.2	1.94
.181	2.604	2.538	...	25.0	2.83	0.11	...	...	...
.227	2.658	...	2.591	...	...	...	25.0	68.2	2.56
.277	2.604	2.538	...	25.0	2.83	0.11	...	...	...
.324	2.631	...	2.564	...	...	...	25.0	68.2	2.59
.375	2.658	2.591	...	25.0	2.83	0.11	...	...	...
.423	2.686	...	2.618	...	...	...	25.0	68.2	2.54
.473	2.658	2.591	...	25.0	2.83	0.11	...	...	...
.523	2.718	...	2.645	...	...	...	25.0	68.2	2.51
.571	2.658	2.591	...	25.0	2.82	0.11	...	...	...
.623	2.718	...	2.645	...	...	...	25.0	68.2	2.51
.669	2.658	2.591	...	25.0	2.82	0.11	...	...	...
.722	2.686	...	2.618	...	...	...	25.0	68.2	2.54
.768	2.686	2.618	...	25.0	2.82	0.11	...	...	...
.823	2.740	...	2.671	...	...	...	25.0	68.2	2.49
.867	2.686	2.618	...	25.0	2.82	0.11	...	...	...
.923	2.718	...	2.645	...	...	...	25.0	68.2	2.51
.965	2.658	2.591	...	25.0	2.82	0.11	...	...	...
.022	2.718	...	2.645	...	...	...	25.0	68.2	2.51
Run 3, Iodine Normality 0.3431, $Ba(OH)_2$ Normality 0.1052[c]									
.093	2.513	...	2.458	...	...	...	25 0	31.2	1.24
.099	2.678	2.623	...	20.0	81.6	3.05	...	...	...
.186	2.513	...	2.458	...	...	...	25.0	31.2	1.24
.200	2.731	2.676	...	20.0	81.6	2.99	...	...	...
.275	2.406	...	2.351	...	...	...	25.0	31.2	1.30
.299	2.678	2.623	...	20.0	81.6	3.05	...	...	...
.364	2.406	...	2.351	...	...	...	25.0	31.2	1.30
.399	2.705	2.650	...	20.0	81.6	3.02	...	...	...
.452	2.380	...	2.325	...	...	...	25.0	31.2	1.31
.499	2.705	2.650	...	20.0	81.6	3.02	...	...	...
.540	2.380	...	2.325	...	...	...	25.0	31.2	1.31
.600	2.731	2.676	...	20.0	81.6	2.99	...	...	...
.625	2.300	...	2.245	...	...	...	25.0	31.2	1.35
.702	2.758	2.703	...	20.0	81.6	2.96	...	...	...
.711	2.326	...	2.271	...	...	...	25.0	31.2	1.34
.803	2.731	2.676	...	20.0	81.6	2.99	...	...	...
.826	2.300	...	2.245	...	...	...	25.0	31.2	1.35

H_2S, %. Tutweiler, 1.14; C.N.G.A., 1.14. CO_2, %. Orsat, 5.46.
H_2S, %. Tutweiler, 0.11; C.N.G.A., 0.11. CO_2, %. Orsat, 2.59.
H_2S, %. Tutweiler, 3.03; C.N.G.A., 3.03. CO_2, %. Orsat, 1.32.

Column 1 is a list of the readings taken on the flowmeter at each change of either the starch or the phenolphthalein indicator. The values are in cubic feet and represent the reading of the meter at the end of each absorption test. No corrections for temperature, barometric pressure, or water content of the gas are included in these values.

The corrected volumes of gas passing through each increment of reagent are recorded in liters in column 2. These values are corrected to the standard conditions customary in the natural gas industry (60° F., 30 inches of mercury, dry) according to the formula:

$$\text{Corrected volume} = \text{observed volume} \times \frac{520}{460 + t} \times \frac{p}{30} \times \frac{p - pw}{p}$$

where

t = observed temperature in ° F.
p = observed barometric pressure in inches of mercury
pw = vapor pressure of water at observed temperature in inches of mercury

These values are further corrected for the acid gas absorbed during the analysis. If gas of rather constant acid gas content is analyzed, this correction is found by calculating the hydrogen sulfide and carbon dioxide absorbed from the total volume of titrating fluid used in the test run and subsequently adding an average prorated volume to each increment. In cases of varying acid gas concentration, the acid gas volume to be added to the discharge volume has to be prorated from increment to increment. The error in both methods is very small and well below the reading error on the meter. Finally, there must be subtracted from the volume read on the meter the gas displaced from each absorber on the addition of titrating fluid. For convenience in computation, the input gas volume is calculated in liters, since all other volume measurements are made in metric units.

The corrected volumes of gas passing through the apparatus for each test are recorded in liters in columns 3 and 4.

Columns 5 and 8 indicate the increments of standard iodine and barium hydroxide solution in milliliters.

Columns 6 and 9 show the calculated volumes of hydrogen sulfide and carbon dioxide corresponding to each increment of titrating fluid:

1 ml. of 0.1 N iodine = 1.183 ml. of hydrogen sulfide at 60° F. and 30 inches of mercury
1 ml. of 0.1 N barium hydroxide = 1.183 ml. of carbon dioxide at 60° F. and 30 inches of mercury

In columns 7 and 10, the hydrogen sulfide and carbon dioxide contents of the gas are listed in volume per cent.

For purposes of comparison, results of analyses by the Tutweiler and C.N.G.A. methods for hydrogen sulfide and by the Orsat method for carbon dioxide are given in footnotes *a*, *b*, and *c*.

If it is desired to express the hydrogen sulfide value in grains per 100 standard cubic feet the percentage has to be multiplied by 628.9.

DISCUSSION

As can be seen from Table I, the results obtained by the proposed method check well with values from analyses by the customary methods. The probable accuracy of the Orsat method, with good equipment is dependent largely on buret reading error. This is stated to be of the order of 0.05 to 0.1 ml. (*8*). Thus, on a gas con-

taining 10% acidic constituents, the percentage error would be ±0.5 to 1.0% on a 100-ml. sample. The C.N.G.A. method is said to be accurate to within 5% (presumably ±5%, *4*), but here again the percentage error is dependent on the total amount of material measured. The accuracy of the Tutweiler method is dependent largely upon the iodine buret reading error. If this error is ±0.05 ml., the use of 5 ml. of iodine solution would give a percentage error of ±1%. Thus it is seen that the method herein described has a probable accuracy of the same order of magnitude as that of the customary methods.

The first value in a series of determinations has to be discarded because some gas is consumed for the saturation of the liquids in the absorption cylinders, which causes erroneous meter readings. Subsequently, the variations between successive determinations should not be greater than ±2.5%. This accuracy holds for a wide range of acid gas concentrations and diminishes only if the acid gas content is very low and very small increments of titrating fluids have to be used. The method is applicable to practically every concentration of hydrogen sulfide and carbon dioxide occurring in natural gas, since accurate analyses can be obtained by appropriate variations of the strength and the increments of the titrating solutions.

The method is readily adapted to routine field testing by measuring the solutions into the absorption cylinders by accurately calibrated pipets, and then passing the gas through the solution until the indicators change color. From the meter readings and the amount of standard solutions used, the acid gas content of such spot samples may be calculated. However, the increments of iodine and barium hydroxide have to be chosen in such a manner that the barium hydroxide is exhausted first in order to avoid erroneous results due to absorption of hydrogen sulfide in the barium hydroxide solution.

CONCLUSIONS

An analytical method has been developed for the continuous and simultaneous determination of hydrogen sulfide and carbon dioxide in a stream of natural gas, using standard iodine and barium hydroxide solutions as titrating fluids.

The results found by this method are accurate and compare favorably with results obtained by methods customary in the gas industry.

Slightly modified, the method can be used for routine work in the field.

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Analysis for Naphthene Ring in Mixtures of Paraffins and Naphthenes

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A new method based on the temperature coefficient of density and either density or refractive index is recommended for the analysis of ring content in mixtures of paraffins and naphthenes. When tested on pure compound data it shows an average deviation of 8% due mainly to isomer effect. The accuracy of the method is dependent to some extent on the degree of condensation of the naphthene rings, and is unsatisfactory for bridged ring naphthenes and single naphthene rings containing more than eight carbon atoms in the ring. The new method is compared with the older methods of Davis and McAllister, and Vlugter, Waterman, and van Westen.

THIS paper continues a general investigation of the interrelationship of physical properties and their use in analytical methods for hydrocarbons (*7*, *9–14*, *17*, *22*) and is concerned with the determination of weight per cent ring carbon atoms in mixtures of paraffins and naphthenes. Per cent ring is arbitrarily defined as 100 times the ratio of the ring carbon atoms to the total carbon atoms, and must be distinguished from per cent naphthenes which is the percentage of naphthenic molecules and includes both ring and side chain.

Two methods for obtaining a naphthene ring analysis have been published. The method developed by Davis and McAllister (*2*) is based on molecular volume and molecular weight and gives number of ring carbon atoms. This method requires two properties, density and molecular weight, and the accuracy is dependent to some extent on the degree of condensation of the naphthene rings. Schiessler *et al.* (*18*) have pointed out the systematic deviation of this equation for high molecular weight hydrocarbons and have worked out a correction for use in this range. This systematic deviation is not surprising in view of the meager pure compound data available in 1930 when the Davis and McAllister paper was published.

The method for per cent naphthene ring developed by Vlugter, Waterman, and van Westen (*20*, *21*) is based on a correlation of Lorentz-Lorenz specific refraction and molecular weight, and requires three properties, density, refractive index, and molecular weight. The use of three physical properties instead of two, as in the Davis and McAllister method, introduces an additional variable involving a knowledge of the size of the naphthene rings (cyclopentane or cyclohexane types). Thus, in the analysis of petroleum fractions by the Waterman method it becomes necessary to make assumptions regarding the size and degree of condensation of the naphthene rings. It is usually assumed that the naphthene rings are condensed cyclohexanes, since for this correlation that type of ring structure best represents the average for all naphthenes.

In the initial stages of this work it was found that there were many combinations of physical properties which would give correlation for determining weight per cent ring. The method presented is based on the temperature coefficient of density and density or refractive index. No other combinations of properties were found which gave a better correlation. The temperature coefficient of density is derived from molecular weight (*13*) which is easily determined from other laboratory inspection tests by means of the correlations of Hirschler (*7*), or Mills, Hirschler and Kurtz (*17*).

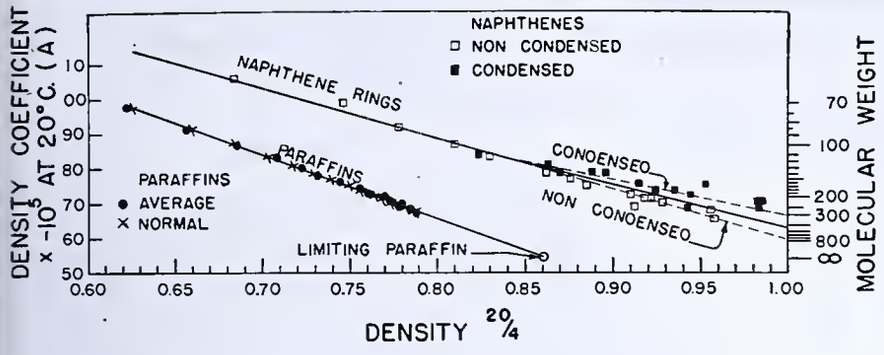


Figure 1

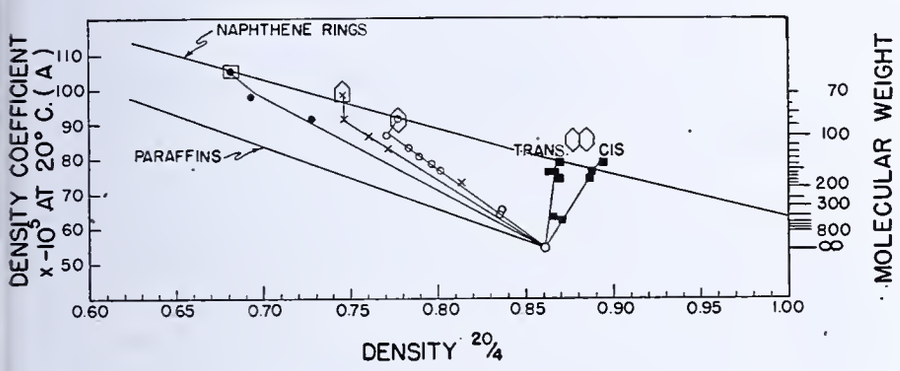


Figure 2

Table I. Relation of Molecular Weight and Temperature Coefficient of Density

Molecular Weight	A^a	Molecular Weight	A^a	Molecular Weight	A^a	Molecular Weight	A^a
70	98.4[b]	92	89.0	140	78.5	260	68.5
71	97.9[b]	94	88.4	143	78.0	271	68.0
72	97.4	96	87.8	147	77.5	284	67.5
73	96.9	98	87.3	150	77.1	297	67.0
74	96.4	100	86.7	153	76.7	310	66.5
75	96.0	102	86.1	156	76.3	324	66.0
76	95.5	104	85.6	159	75.9	340	65.5
77	95.0	106	85.1	163	75.5	357	65.0
78	94.5	108	84.6	167	75.0	374	64.5
79	94.1	110	84.1	172	74.5	394	64.0
80	93.6	112	83.7	177	74.0	415	63.5
81	93.2	114	83.2	182	73.5	439	63.0
82	92.8	116	82.8	187	73.0	467	62.5
83	92.3	118	82.4	193	72.5	499	62.0
84	91.9	120	82.0	199	72.0	538	61.5
85	91.5	122	81.6	205	71.5	586	61.0
86	91.1	125	81.0	213	71.0	643	60.5
87	90.7	128	80.5	221	70.5	718	60.0
88	90.4	131	80.0	229	70.0	805	59.5
89	90.0	134	79.5	239	69.5	902	59.0
91	89.4	137	79.0	249	69.0		

[a] $A = -10^5 \times \alpha$ (temperature coefficient of density at 20° C.).
[b] These values are probably less accurate than the others.

DERIVATION OF EQUATIONS

It was found on plotting density versus the temperature coefficient of density for paraffins and noncondensed naphthene rings containing no chain (Figure 1), that linear relationships exist for each of these two classes of hydrocarbons. A third straight line branching from the noncondensed naphthene ring line can be drawn through the data for condensed naphthene rings.

As shown in Figure 2, the addition of a side chain to a naphthene ring causes the properties to approach those of a paraffin of infinite molecular weight and the degree of approach is roughly proportional to the percentage of chain. The density of the paraffin of infinite molecular weight, sometimes known as the limiting paraffin, is 0.861 (*10*). For mixtures of paraffins and naphthenes in the region below 0.861 density, an equation was derived assuming the percentage of paraffin plus paraffin side chain can be obtained by interpolation between the paraffin line and the naphthene ring line, along a line of constant density. Thus, for $d < 0.861$

$$\text{Weight \% ring} = \frac{A + 190.0d - 217.9}{0.593d - 0.249} \quad (1)$$

where

d = density at 20° C.

A = $-10^5 \times$ temperature coefficient of density (α) derived from molecular weight (see Table I)

Figure 3 demonstrates that this method of interpolation is satisfactory for naphthenic hydrocarbons; the deviations of the calculated from the experimental weight per cent ring for the alkyl cyclohexanes and alkyl cyclopentanes are shown. The greatest deviations seem to be in the initial members of the series; this irregularity is common to most physical properties of these hydrocarbons.

Table II demonstrates that the method is also satisfactory for mixtures of paraffins and naphthenes. In three examples hypothetical properties for binary mixtures of a paraffin and a naphthene at several different concentrations have been calculated. Hydrocarbons which agree fairly well with the correlation were chosen for these calculations. Two sets of the mixtures shown consist of hydrocarbons which would not ordinarily be encountered together, but the results on these serve to indicate that the method is useful for samples of wide boiling range.

In the region above 0.861 density, saturated petroleum fractions consist mainly of naphthenes as shown by the work of Mair, Willingham, and Streiff (*16*), which indicates that paraffins do not exist in lubricating oils. A second equation was derived for this region, assuming that the percentage of paraffin plus paraffin side chain can be obtained by interpolation between the naphthene ring line and the limiting paraffin point along a straight line drawn through the point representing the sample. Thus, for $d > 0.861$

$$\text{Weight \% ring} = \frac{A + 102.8d - 142.8}{0.262} \quad (2)$$

Above 0.861 density, the naphthene ring line is a compromise line midway between the condensed and noncondensed naphthene rings. This assumes an equal distribution of the condensed and noncondensed types and introduces an uncertainty which becomes larger with increasing number of rings per molecule. If one type predominates in the tri- and tetracyclic range, the error may be as great as 5 to 10%. When our state of knowledge is sufficiently advanced that we know the relative amounts of con-

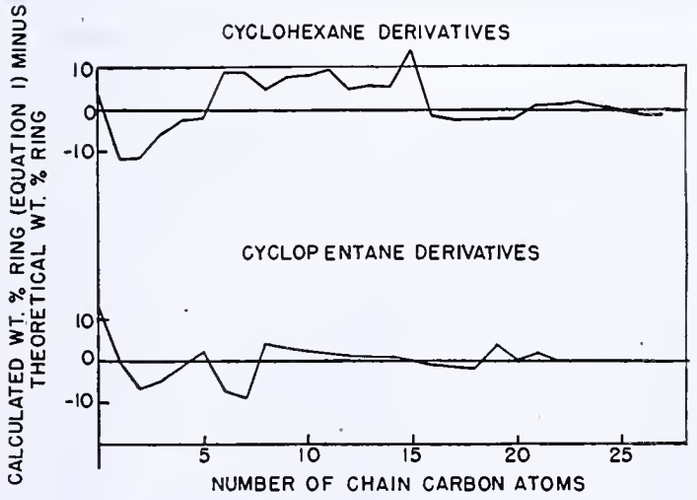


Figure 3

Table II. Agreement of Calculated with Theoretical Weight Per Cent Naphthene Ring for Hypothetical Binary Mixtures

Hypothetical Mixtures	Mole % of Paraffin	Molecular Weight	d_4^{20} [a]	A	% Ring Carbons		
					Caled.	Theoretical	Deviation
1-Methyl-2-amyl cyclohexane ($C_{12}H_{24}$) with 5-butylnonane ($C_{13}H_{28}$)	0	168.3	0.8146	74.9	50	50	0
	20	171.5	0.8028	74.5	40	39	1
	40	174.8	0.7917	74.2	30	29	1
	60	177.9	0.7814	73.9	21	19	2
	80	181.2	0.7717	73.6	11	9	2
	100	184.4	0.7624	73.3	1	0	1
2-Ethyldecalin ($C_{12}H_{22}$) with 3,3-dimethylpentane (C_7H_{16})	0	166.3	0.8702	75.1	83	83	0
	25	149.8	0.8346	77.1	72	70	2
	50	133.2	0.7940	79.6	57	53	4
	75	116.7	0.7474	82.7	35	30	5
	100	100.2	0.6933	86.6	2	0	2
Cyclohexane (C_6H_{12}) with n-dodecane ($C_{12}H_{26}$)	0	84.2	0.7786	91.8	102	100	2
	20	101.4	0.7683	86.3	70	67	3
	40	118.6	0.7612	82.3	45	43	2
	60	135.9	0.7559	79.2	25	25	0
	80	153.1	0.7519	76.7	9	11	−2
	100	170.3	0.7488	74.7	−5	0	−5

[a] Densities calculated on volume % basis assuming no volume change on mixing.

densed and noncondensed naphthenes in different petroleum fractions, we can replace Equation 2 with two separate equations for condensed and noncondensed naphthene rings.

A plot similar to the density versus A correlation may be derived by plotting refractive index against A, and an alternative set of equations have been derived in terms of these properties

For $n < 1.478$

$$\text{Weight \% ring} = \frac{A + 374.1n - 607.2}{1.152n - 1.471} \qquad (1A)$$

For $n > 1.478$

$$\text{Weight \% ring} = \frac{A + 232.9n - 398.4}{0.232} \qquad (2A)$$

Where n = refractive index at 20° C. for the D line of sodium.

The authors recommend use of Equations 1 and 2 derived in terms of temperature coefficient of density and density. Equations 1A and 2A appear to be slightly less accurate, but may be used as an alternative when it is more convenient to obtain refractive index than density. Inspection of these equations indicates that weight per cent ring can also be calculated from density and refractive index alone. This calculation has been found to give considerably greater error and is not recommended.

The four equations were derived from the physical property data on the normal paraffins and naphthene rings without side chain. A slight adjustment, however, was necessary to obtain a better fit with the data for the whole mass of paraffins and naphthenes. Therefore the final equations do not agree exactly with the lines shown in Figures 1 and 2. The data on pure hydrocarbons were assembled from the compilations of Doss (*3*), Ward and Kurtz (*22*), Egloff (*5*), Eaton (*4*) and A.P.I. Research Projects No. 42 (*18*, *24*) and No. 44 (*1*, *19*).

ACCURACY

Equations 1, 1A, 2, and 2A have been tested on pure compound data for the paraffins and the mono- and polycyclic cyclopentanes and cyclohexanes (Table III). The average deviation is 8%. The data on bridged ring compounds and rings containing more than six carbon atoms have been excluded from Table III because the bridged ring compounds show more than the true amount of ring as demonstrated in Table IV, and single rings containing more than eight carbon atoms show less than the true amount of ring as demonstrated in Figure 4; which contains data for single-ring saturated hydrocarbons without side chain from 5 to 34 carbon atoms.

As shown in Figure 4, the calculated per cent ring decreases with increasing ring size. The calculated percentage of ring drops to 30% for a single-ring naphthene containing 25 carbon atoms and no side chain. For rings larger than 25 carbon atoms there does not appear to be any further drop in the calculated percentage of ring. This may mean that large rings tend to collapse and become more chainlike in nature.

In the analysis of petroleum fractions we need not expect too much trouble from these anomalies, since rings larger than six carbon atoms have not yet been isolated from petroleum, and the

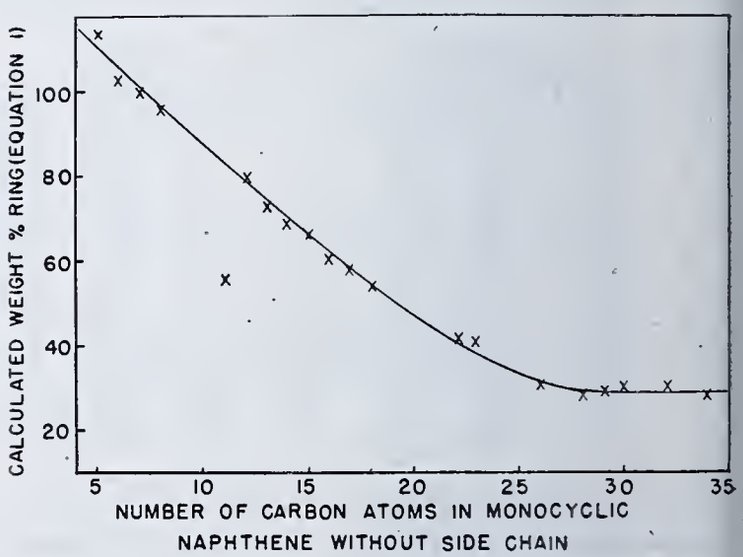


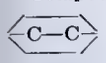
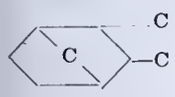
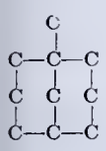
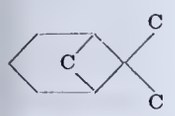
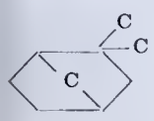
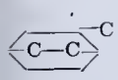
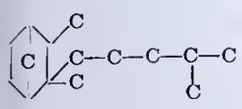
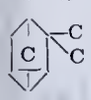
Figure 4

Table III. Comparison of Methods for Pure Compound Data

Hydrocarbon Types	No. of Compounds	Calculated Weight Per Cent Ring: Average Deviation [a]				Deviation of Average [b]				Maximum Deviation			
		A–d [c]	A–n [d]	Davis	Waterman	A–d	A–n	Davis	Waterman	A–d	A–n	Davis	Waterman
Paraffins	224	7	7	9	6	0	0	6	3	−55	−68	−63	121
Monocyclics													
C_5 ring	56	6	9	11	5	−2	−7	−7	−2	−19	−27	−20	−14
C_6 ring	115	8	9	10	11	1	1	−1	−10	+28	+44	−91	+51
Dicyclics noncondensed													
C_5 rings	7	2	5	6	6	−1	−4	−2	−4	−6	+27	−15	−9
C_6 rings	26	5	7	7	21	−4	2	2	−17	−13	+16	39	51
Mixed C_5 and C_6 rings	7	6	6	6	15	−6	−6	−6	−15	−13	+31	−11	−19
Dicyclics condensed													
C_5 rings	4	8	10	8	13	6	1	1	10	+14	+12	−12	19
C_6 rings	32	6	5	9	8	5	6	8	−7	+18	+37	25	−51
Mixed C_5 and C_6 rings	7	8	6	7	10	8	5	7	4	+12	+9	10	16
Tricyclics	13	10	10	9	18	−3	−2	4	−12	+25	+39	36	−41
Tetracyclics	6	10	9	8	19	−6	0	5	−17	−14	+18	22	−59
Average (according to No. of compounds)		7	8	8	12	0	0	2	−6	..	..	..	..

[a] Without regard to sign. [b] Taking sign into account. [c] (A–d) refers to calculation by Equations 1 or 2. [d] (A–n) refers to calculation by Equations 1A or 2A.

Table IV. Comparison of Methods for Saturated Bridged Ring Compounds

Compounds	Deviation of Calculated Weight % Ring			
	A–d	*A*–*n*	Davis	Waterman
	14	11	12	8
	25	22	27	24
	−7	−16	18	14
	25	20	31	28
	9	−1	38	35
	23	20	31	29
	14	11	25	24
	29	25	53	52
	24	5	81	78
	23	18	54	54

bridged ring structures which have been shown to be present are probably in small concentration in most crudes.

As shown in Table III, accuracy of the recommended method is only slightly better than obtained with the Davis-McAllister and Waterman methods when compared on the basis of the whole mass of pure compound data. (The Waterman method in this case has been used, assuming the condensed cyclohexane type of ring structure is the predominant polycyclic naphthene type.) However, a comparison on hydrocarbon fractions of higher molecular weight shows a pronounced difference between the recommended method and these other methods. In general, the Davis-McAllister method gives results which are on the average as much as 10% higher than the *A*-density method, and the Waterman method gives results which are as much as 5% lower (see Tables V, VI, VII). Work done by A.P.I. Project No. 42 (*18*, *24*) shows that the *A*-density method is accurate within 2 to 3% on the pure compounds of high molecular weight. This indicates the recommended method is probably the most accurate in the high molecular weight range.

The Davis and McAllister method and the *A*-density method both use the same physical properties, molecular weight and density, so that it is not reasonable to believe that one would be subject to more variables than the other. It is only because the present correlators had more and better data that the new correlation shows less deviation.

An example of the accuracy of this method for unusual materials is demonstrated on three petroleum wax fractions originally reported by Ferris, Cowles, and Henderson (*6*), and later analyzed for carbon and hydrogen content by Mair and Schicktanz (*15*). A comparison is made of weight per cent ring calculated from Equation 1 with values calculated from carbon and hydrogen content, assuming a variety of possible naphthene ring structures in mixtures with paraffin, where the carbon and hydrogen value show naphthene to be present. As shown in Table VIII, the agreement is good.

In the routine use of this method for the high melting point waxes, weight per cent ring can be determined from melting point and refractive index in the liquid state, usually at 80° C. The molecular weight is then calculated from refractive index and melting point by the method of Mills, Hirschler, and Kurtz (*17*), and the refractive index is corrected to 20° C. using Equations 1 and 2 (*13*). The latter equation is sufficiently accurate for the purpose of this paper, although it is not in general recommended for correcting refractive index over a wide range of temperature. The application of this type of analysis to aromatic rings is being studied.

ACKNOWLEDGMENT

The statistical data contained in this paper have been completely revised since its presentation at the 1941 Atlantic City meeting of the American Chemical Society. The authors wish to acknowledge the assistance of H. P. Spain in carrying out many of the calculations.

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Table V. Comparison of Methods for Hydrogenated Lube Oil Extracts of Mair, Willingham, and Streiff (*16*)

Fractions	Weight % Ring *A*–d	Deviation from *A* – d Method *A*–*n*	Davis	Waterman
B-12-H	74	3	10	−5
B-16-H	72	3	10	−6
B-19-H	70	3	5	−7
C-1-H	78	4	10	−3
C-7-H	77	3	13	−5
C-13-H	75	3	11	−5
C-20-H	69	3	11	−5
C-(26 + 27)-H	60	3	11	−4
C-30-H	56	2	10	−6
C-33-H	51	2	9	−6
C-35-H	47	2	7	−7
C-37-H	43	2	7	−10
C-res-H	34	0	4	−10
E-1-H	75	4	12	−7
E-25-H	50	3	12	−4
Deviation of average		3	10	−6

Table VI. Comparison of Methods for Hydrogenated Olefin Polymers of Waterman, Leendertse, and Makkink (23)

Fractions	Weight % Ring *A*–d	Deviation from *A*–d Method *A*–*n*	Davis	Waterman
II	6	−1	5	0
IV	5	0	7	−2
I′	11	1	7	−4
II′	13	1	6	−4
III′	12	1	6	−4
IV′	17	1	5	−6
V′	16	0	6	−5
VI′	18	1	6	−7
Deviation of average		1	6	−4

Table VII. Comparison of Methods for Hydrogenated Gas Oils of Kreulen (8)

Fractions	Weight % Ring *A*–d	Deviation from *A*–d Method *A*–*n*	Davis	Waterman
1	68	−4	−1	−8
2	66	−2	2	−4
3	65	0	5	−4
4	47	−5	1	−5
6	49	−5	1	−5
5	42	−4	2	−6
11	43	−3	−6	−8
7	45	−5	1	−5
8	31	−3	3	−4
9	28	−3	4	−2
10	33	−3	3	−5
Deviation of average		−3	1	−5

Table VIII. Accuracy of Calculation for Three Wax Fractions Refined by Ferris *et al.* (6)

	Weight Per Cent Ring				
	Calculated from Empirical Formula[a] Determined by Mair and Schicktanz (*15*), Assuming Mixtures of Paraffins with Naphthenes of Following Types:				
Fraction	Calcd. by Equation 1	One or more cyclohexane rings per molecule	One or more groups per molecule	One or more cyclopentane rings per molecule	One or more groups per molecule
6A	−4[b]	−2[b]	−2[b]	−2[b]	−2[b]
6I	9	14	11	11	9
6J	12	15	13	13	10

[a] Mair and Schicktanz analyzed samples prepared by Ferris, Cowles, and Henderson after these had been in storage for some time. A correction to x in formula C_nH_{2n+x} was made to allow for loss of hydrogen due to slight oxidation while in storage. Mair and Schicktanz state that they have probably overcorrected for this loss of hydrogen, which would result in slightly low values for the weight per cent ring calculated from empirical formula.

[b] Hypothetical negative value for per cent ring is reported for a strict comparison of accuracy. In practice, negative values for calculated weight per cent ring would be reported as 0.

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Presented before the Division of Petroleum Chemistry at the 102nd Meeting of the American Chemical Society, Atlantic City, N. J.

Equation Relating Density, Refractive Index, and Molecular Weight for Paraffins and Naphthenes

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An equation is presented relating refractive index for the sodium *D* line, density, and temperature coefficient of density, each at 20° C., for paraffins, naphthenes, and mixtures of saturated hydrocarbons in the liquid state. The temperature coefficient of density at 20° C. is obtained from molecular weight using the correlation of Lipkin and Kurtz (9). The equation correlates density and refractive index for all saturated hydrocarbons more accurately than previous equations, but is not accurate for the calculation of molecular weight. It may be applied to hydrocarbon mixtures, such as saturated petroleum fractions, because it does not require a knowledge of molecular formula or structure. Refractive index may be calculated from density and the temperature coefficient of density with an accuracy of ±0.002 for pure hydrocarbons and ±0.001 for petroleum fractions. Likewise, density may be calculated from refractive index and the temperature coefficient of density with an accuracy of ±0.004 and ±0.002, respectively. The equation provides the basis for further correlations which will serve as tools in the analysis of hydrocarbon mixtures.

THIS paper is part of a general investigation (*6–11*) of the relationship between density, refractive index, and other physical properties of hydrocarbons and the relationship of these properties to hydrocarbon structure.

Lipkin and Kurtz (*9*) have shown that the variation of density, refractive index, and the temperature coefficient of density with temperature is related to molecular weight. More recently Lipkin, Martin, and Kurtz (*11*) demonstrated in Figure 1 of their paper the relationship of density at 20° C. and the temperature coefficient of density at 20° C. to hydrocarbon structure. Figure 1 of this paper shows the similar relationship for refractive index and the temperature coefficient of density. From these two correlations which differ in only one variable, density in the first and refractive index in the second, it was possible to derive Equation 1 for calculating refractive index from density and the temperature coefficient of density.

$$n = \frac{69.878\mathrm{d} - 0.4044A\mathrm{d} - 0.797A + 136.566}{5.543\mathrm{d} - 0.746A + 126.683} \quad (1)$$

For the calculation of density this relation may be put in the form of Equation 2.

$$\mathrm{d} = \frac{126.683n - 0.746An + 0.797A - 136.566}{-5.543n - 0.4044A + 69.878} \quad (2)$$

In these equations

n = refractive index at 20° C. for the *D* line of sodium
d = density at 20° C.
A = $-10^5 \times$ temperature coefficient of density (α), which is obtained from the approximate molecular weight (Table I, *11*)

The derivation was originally based on data for eight *n*-paraffins and fourteen monocyclic and noncondensed polycyclic naphthenes without side chains. Slight adjustments were then made to obtain better agreement with the average data on almost 600 hydrocarbons.

Since accurate determination of the temperature coefficient of

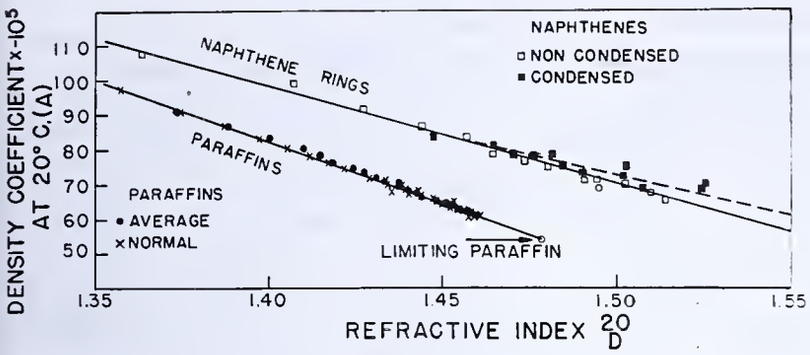

Figure 1

density is difficult, this property is derived from molecular weight (*9*). The average molecular weight for mixtures of hydrocarbons may be obtained experimentally, or from correlations with other physical properties. In this laboratory, molecular weights are obtained either from the boiling point–density correlation of Mills, Hirschler, and Kurtz (*14*) or from the correlation of Hirschler (*4*) using the viscosities at 100° and 210° F.

Approximate molecular weights are adequate for the calculation of refractive index or density especially in the molecular weight range above 100. The effect of a 5% error in the molecular weight on the calculation of refractive index is shown in Table I. The data are calculated for paraffins (*6*). The error in the calculated refractive index decreases from 0.0014 at 70 molecular weight to 0.0002 at 400 because of the asymptotic nature of the relation (*9*) between molecular weight and temperature coefficient of density.

COMPARISON WITH OTHER EQUATIONS

Equation 1 differs from other equations for the calculation of refractive index from density and molecular weight in that it does not contain constants the evaluation of which requires a knowledge of molecular structure.

Table II presents a comparison of the accuracy of density and refractive index values for the C_5–C_9 isoparaffins calculated by Equations 1 and 2 and by the method of Taylor, Pignocco, and Rossini (*15*). The latter method requires a knowledge of the exact structure of the isoparaffins and the calculation of the hydrocarbon's density and refractive index is based on the values of those properties for the normal isomer. The data indicate that this method is slightly more accurate for the C_5–C_8 isoparaffins but that Equations 1 and 2 are slightly better for the C_9 isoparaffins.

Kurtz *et al.* (*7*, *8*) have discussed the relative accuracy of the various equations relating refractive index and density. Comparison of the accuracy of Equation 1 with that of the older equations of Newton, Gladstone and Dale, Sellmeier-Drude, and Eykman as applied to the calculation of the refractive index of the isomeric C_7 paraffins is given in Table III. For these older equations the authors have used (1) specific refraction constants calculated from the average properties of the whole group of C_7 paraffin isomers, (2) Lorentz-Lorenz specific refractions calculated from both the average properties of the isomers and from the Eisenlohr atomic refractions, and (*3*) the constants developed by Kurtz and Lipkin (*7*) for the Sellmeier-Drude equation. The data in Table III demonstrate that only the Newton and Sellmeier-Drude equations provide results comparable in accuracy with Equation 1.

The deviations between the best literature values for refractive index and refractive index calculated by a particular equation for a group of isomers can sometimes be correlated with a particular physical property. In several of the older equations this deviation apparently varies with density; the deviations obtained by using Equation 1 seem to vary with the aniline points of the hydrocarbons. The authors have no explanation for these facts at present.

Table I. Difference in Calculated Refractive Index of Paraffins, Assuming 5% Error in Molecular Weight

Molecular Weight			
True	Assumed	d_4^{20}	Δn
70	73.5	0.623	0.0014
100	105.0	0.686	0.0008
150	157.5	0.738	0.0005
200	210.0	0.765	0.0004
300	315.0	0.796	0.0003
400	420.0	0.810	0.0002

EXPERIMENTAL DATA

Equations 1 and 2 have been tested with data for a large number of paraffins and naphthenes and saturated fractions from petroleum.

The pure hydrocarbon data are identical with those used previously (*11*). Data on petroleum fractions include the hydrogenated cracked paraffin waxes of Kreulen (*5*), the hydrogenated polymerized pentenes of Waterman (*18*), the acid-treated oils of Bestushew (*2*, *17*), the hydrogenated and solvent-extracted oils of Mair (*12*, *13*), and the hydrogenated oils of Vlugter *et al.* (*16*). Data were obtained in this laboratory on virgin gasolines, gas oils, and fractions of a catalytically cracked gas oil, all acid-treated to remove aromatics and olefins.

Table II. Comparison of Methods for Calculating Density and Refractive Index for Isoparaffin Data

	Deviation of Calculated Properties			
	$d_4^{20} \times 10^4$		$n_D^{20} \times 10^4$	
	Equation 2	N.B.S. method (*15*)	Equation 1	N.B.S. method (*15*)
	C_5 to C_8 Isomers[a]			
Average deviation	11	7	4	4
Maximum deviation	42	24	17	11
	C_9 Isomers[b]			
Average deviation	13	15	6	
Maximum deviation	40	38	16	22

[a] Six C_5–C_8 isoparaffins not included in tabulation because four normal isomers are used as reference standards in N.B.S. method, neopentane is a gas at 20° C., and 2,2,3,3-tetramethylbutane is a solid at 20° C.
[b] *n*-Nonane, 2,2,3,3-tetramethylpentane, and other nonanes for which (*1*) does not list accurate data excluded from tabulation.

The sources of the molecular weights necessary for the calculation of the temperature coefficient of density were as follows: pure hydrocarbons, theoretical values; oil fractions reported in the literature, experimental values in the reference; gasoline and gas oil fractions from the authors' laboratory, from the density–boiling point relationship of Mills, Hirschler, and Kurtz (*14*).

ACCURACY

The accuracy with which refractive index and density can be calculated by Equations 1 and 2 is demonstrated in Table IV, where the deviations of the calculated from the experimental values for the entire mass of data are tabulated. Calculated refractive indices show an average deviation (without regard to sign) of 0.002 for pure compounds and 0.001 for petroleum fractions. Calculated densities show an average deviation of 0.004 gram per ml. for pure compounds and 0.002 gram per ml. for petroleum fractions. In general, the properties of petroleum fractions may be calculated much more accurately than those of pure compounds because in a mixture of hydrocarbons there is a tendency toward cancellation of the isomer and molecular structure effects found in calculations involving the individual hydrocarbons.

Table III. Comparison of Equations for Calculation of Refractive Index of Isomeric Heptanes

(Calculated − Observed n_D^{20}) × 10^4

	Equation 1	Newton, $\frac{n^2-1}{d} = C^a$	Gladstone and Dale, $\frac{n-1}{d} = C^a$	Eykman, $\frac{n^2-1}{n+0.4} \times \frac{1}{d} = C^a$	Lorentz-Lorenz, $\frac{n^2-1}{n^2+2} \times \frac{1}{d} = C$		Sellmeier Drude[c]
					C^a	C^b	
2,4-Dimethylpentane	12	0	−10	−12	−19	−11	−2
2,2-Dimethylpentane	10	−2	−11	−15	−19	−11	−4
2-Methylhexane	4	−5	−10	−12	−15	−8	−7
n-Heptane	−4	−9	−10	−13	−11	−4	−11
3-Methylhexane	−1	−3	−2	−1	−1	7	−5
2,2,3-Trimethylbutane	3	4	7	9	11	18	2
3,3-Dimethylpentane	2	5	11	13	16	24	3
2,3-Dimethylpentane	0	4	11	16	18	26	1
3-Ethylpentane	−2	5	15	19	23	32	2
Av. deviation	4	4	10	12	15	16	4
Deviation of av.	3	0	0	0	0	8	−2
Maximum deviation	12	9	15	19	23	32	−11

[a] C calculated from average properties for isomeric heptanes: $d_4^{20} = 0.6859$ and $n_D^{20} = 1.3879$ (*15*).
[b] C calculated from Eisenlohr atomic refraction constants.
[c] Using constants for average paraffins developed by Kurtz and Lipkin (*7*).

Table IV. Accuracy of Calculated Densities and Refractive Indices

	Number	Refractive Index $n_D^{20} \times 10^4$ Av. dev.	Dev. of av.	Max. dev.	Density $d_4^{20} \times 10^4$ Av. dev.	Dev. of av.	Max. dev.
Pure compounds							
Paraffins	230	10	−4	46	22	6	−108
Monocyclics	217						
C_3 ring	15	28	11	73	68	−28	179
C_4 ring	10	20	10	48	48	−26	−116
C_5 ring	56	18	15	52	43	−37	−114
C_6 ring	111	20	−13	−138	47	30	329
C_7 ring	5	26	−26	141	65	65	141
C_8–C_{34} rings	20	41	−41	−109	98	96	263
Dicyclics	105						
Noncondensed	43	26	−17	−93	64	40	223
Condensed	46	23	+1	−156	56	−2	223
Bridged rings	16	31	+31	+121	70	−70	−291
Tri- and tetracyclics	24	40	22	+126	95	−69	−304
Av.	576	19	−3	−156	44	5	−304
Petroleum fractions							
Lubricating oil	78	11	+3	19	26	−7	−50
Gas oil	24	2	0	−6	5	−1	−15
Gasoline	7	3	+2	+6	9	−8	−15
Av.	109	9	+2	19	20	−6	−50

The accuracy with which molecular weight may be calculated from Equation 1 (by calculating temperature coefficient of density at 20° C. and converting to molecular weight by means of Table I, *11*) has been investigated. The magnitude of deviations increases rapidly with increasing molecular weight, and may be ascribed to the rapidly increasing $\frac{\Delta \text{ molecular weight}}{\Delta\alpha}$. The equation is not satisfactory for the calculation of molecular weight, especially when the molecular weight is greater than 150.

The accuracy of calculations by these equations is nearly independent of the structure of the saturated hydrocarbons, but there are a few noticeable trends. These are difficult to evaluate quantitatively because the experimental inaccuracies of the data are frequently of the same order of magnitude as the deviations in the calculation.

The qualitative discussion of trend is confined to the refractive index calculations as shown in Table IV. The paraffin hydrocarbons have the smallest deviation of all classes. Monocyclic naphthenes show an increasing negative deviation with increasing ring size; the deviation of the average (taking sign into account) of the alkylcyclopentanes is +0.0015 and the alkylcyclohexanes is −0.0013. Since petroleum fractions contain both cyclopentane and cyclohexane derivatives (*3*, *10*), the deviations tend to cancel each other. The condensed dicyclic naphthenes show no trend, but the noncondensed dicyclics show a systematic deviation of −0.0017.

The deviations of calculated density are approximately twice as large as the deviations of calculated refractive index, and of opposite sign. The larger deviation for the density calculation may be explained by the fact that the numerical difference in density between paraffins and naphthenes is approximately twice as large as the difference in refractive index.

Many of the deviations in the calculated densities and refractive indices may be attributed to errors in the reported properties of the pure hydrocarbons. These errors are frequently of the order of ±0.001 and may be due to impure products, inaccurate determination of properties, or the determination of properties on two different samples, not necessarily of the same purity. It has been frequently observed that physical properties calculated by Equations 1 and 2 for a set of hydrocarbon fractions from some one laboratory show small systematic deviations from the experimental values. This may be attributed to a predominant molecular structure type in these fractions or to the fact that the experimental precision of the data is better than the accuracy.

By means of the equation shown in this paper and the correlations shown in (*9*) it is possible to correlate at any temperature, where the hydrocarbons are in the liquid state, the density, refractive index, and temperature coefficient of density for saturated hydrocarbons. The significance of the application of this equation to olefins and aromatics will be discussed in a subsequent paper.

ACKNOWLEDGMENT

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Automatic Apparatus for Determination of Small Concentrations of Sulfur Dioxide in Air

New Countercurrent Absorber for Rapid Recording of Low and High Concentrations

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A simple countercurrent absorber is described in which practically complete absorption of the water-soluble gases such as sulfur dioxide, ammonia, and hydrogen chloride may be obtained with gas flow rates ranging from less than 10 to more than 5000 times the liquid rates. This absorber, when used in conjunction with a conductivity flow cell of small volume, gives a rapid recording of the concentration from a few parts per billion to several per cent. With the lower gas concentrations, the record is a running average, covering 1 to 2 minutes. An alternative absorber containing conductivity electrodes can reduce this time to 10 to 15 seconds, but absorption is not complete. A simple pump for delivering small volumes of liquid is also described. Equipment embodying this absorber has been applied to the measurement of atmospheric contamination, to the chemical control of a pilot plant making elemental sulfur from sulfur dioxide, and to the rapid measurement of sulfur dioxide in flue gases in the presence of carbon dioxide.

IN THE automatic apparatus for the determination of small concentrations of sulfur dioxide in air previously described (*6–9*), a uniform air stream is aspirated through a measured volume of absorbing solution for a definite time, after which the air stream is changed to a second absorber that has been automatically charged with fresh solution while the first absorber was aspirating. The sulfur dioxide is oxidized to sulfuric acid by hydrogen peroxide, and the course of the absorption is followed by measuring continuously the conductivity of the solution on a recording Wheatstone bridge. The record thus gives primarily the average gas concentration over a given time period, but it also shows a considerable part of the variability of the concentration during that period, represented by differences in the slope of the conductivity curve.

It has been recognized that a more or less "instantaneous" method of recording the gas concentration would be advantageous in many applications, though the average values of short intervals (20 to 30 minutes) are indispensable for any practicable summation of the record over long periods of time. Such instantaneous recordings have heretofore been precluded by lack of a suitable absorber to handle the large ratio of gas volume to liquid volume (about 5000 to 1) required for this analysis.

APPARATUS

Starting with an observation of one of the authors (J.O.I.) that the movement of gas and liquid in opposite directions in a small tube is greatly facilitated by inserting into the tube a pair of twisted-together wires, absorbers have been constructed utilizing the principle of countercurrents, which fulfill the following conditions: (1) The gas and liquid move in opposite directions without bubbling. (2) The walls of the absorber are uniformly wet by liquid which flows in a continuous capillary stream following the wire, from one end of the absorber to the other without forming isolated wet or dry spots. (3) Absorption of sulfur dioxide is practically complete in any range of concentration, from a fraction of a part per million to several per cent, depending on the dimensions of the absorber. (4) The conductivity of effluent liquid can be determined continuously in a small flow cell to give a running, short-time, average measure of the gas concentration.

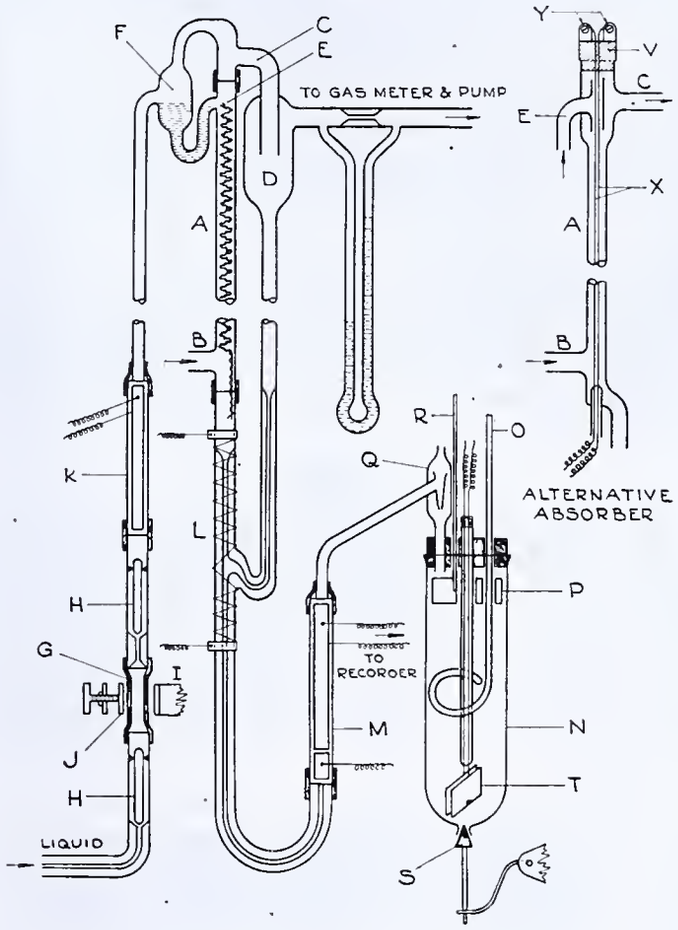

Figure 1. Diagram of Field Sulfur Dioxide Autometer

It is shown below that the indicated concentration at any moment is an average value covering a period which may be as long as 1 or 2 minutes, following the lag representing the time required for the liquid to flow from the absorber to the cell. In an alternative, less efficient absorber, the total time for the analysis may be reduced to 10 to 15 seconds.

Figure 1 is a diagram and Figure 2 is a photograph of an assembly designed to determine sulfur dioxide in the atmosphere in the concentration range up to about 3 to 5 p.p.m., with provision for automatically increasing this range severalfold if necessary. The absorber, *A*, is a 730-mm. length of glass tubing 7.6 to 7.8 mm. in inside diameter, with T-connections near each end. The tube is packed with a helix consisting of two strands of No. 26 B. & S. gage wire, twisted loosely together, then coiled into a 6-pitch spiral which fits snugly against the inside wall. The wire preferably should be of platinum, but Nichrome will withstand a long period of operation before it is seriously corroded, if the hydrogen peroxide concentration is not too high.

The air sample enters the absorber through *B* and leaves at *C*. A convenient rate is 12 to 15 liters per minute. The absorbing solution (2 to 4 $\times$ 10^{-3} *M* hydrogen peroxide in 5 $\times$ 10^{-5} *N* sulfuric acid) is pumped into the tube at *E* at the rate of 3.3 ml. per minute. Trap *D* is provided in case bubbling should occur which would carry part of the liquid over with the gas. A satisfactory liquid pump is obtained by squeezing slightly at 1-second intervals a piece of 6.3-mm. heavy-walled rubber tubing, *G*, mounted

between two glass check valves, H. Delivery from the pump is controlled by adjusting stop J against the movement of plunger I. The reservoir, F, eliminates most of the pulsation in the flow. A stainless steel Zenith gear pump, such as is used in the rayon industry, also gives an excellent controlled flow of liquid, but unfortunately the metal is slightly attacked by the solution.

The solution, after emerging from the absorber, is warmed to room temperature by low-voltage current through a resistance wire on the tube at L. This also reduces the amount of dissolved gas in the liquid. The solution flows through a 2-mm. capillary U-tube into the conductivity cell, M, which has two platinum plates, 5 × 75 mm., mounted parallel in polystyrene with a spacing of 1.8 mm. and a capacity of 0.7 ml. One of the plates may be cut into two pieces, one piece, say, four times the length of the other, and provided with separate leads in order to increase, if necessary, the measurable conductivity range by disconnecting part of the plate area. The liquid then flows to an accumulating vessel, N, in which 100 ml. are collected and well mixed by the stirrer, O. When vessel N is full, the polystyrene float, P, operates an electric switch through the rod, R, starting a clock mechanism that first transfers the recorder from cell M to electrodes T for 1.5 minutes; then transfers the recorder to cell K for 1.5 minutes for a zero reading, at the same time emptying N by operating a solenoid to pull out the rubber plug, S, for a few seconds; and finally returns the recorder to cell M for about 27 minutes before repeating these operations. The rate of liquid flow is thus indicated, since the amount of solution required to operate the float is constant. The rate of gas flow is recorded as in former machines by a contact on the 1 cu. foot dial of the gas meter. The three cells, K, M, and T, have nearly identical calibration curves. The record, therefore, combines a running, short-time, average concentration with a half-hourly average concentration.

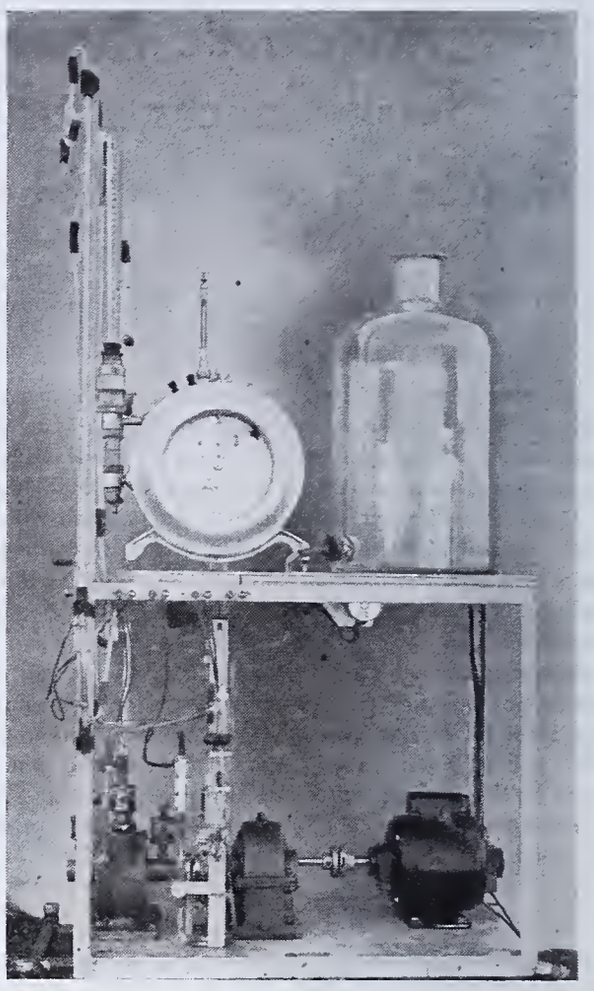

Figure 2. Field Sulfur Dioxide Autometer

EFFICIENCY

The completeness of absorption of sulfur dioxide in this absorber has been determined by operating it in series with an effective bubbler tube and observing the conductivity change in the latter. The following efficiencies were noted at 15 to 18 liters per minute: 2 p.p.m., 98 to 99%; 5 p.p.m., 98%; 15 p.p.m., 96%; and 24 p.p.m., 95%. A tube 350 mm. long absorbed about 80% of the gas under similar conditions. The water flow was 3.3-ml. per minute in all experiments, unless otherwise specified.

If the wire spiral in the 730-mm. tube was spread out to 4-pitch, so that definite dry spaces were maintained between the adjacent capillary streams along the wire, the efficiency fell to 90 to 95%. Efficiency was reduced by twisting the wires too tightly together; a pair of wires with the twists spaced at 1 mm. was slightly less efficient than a pair with twists 3 mm. or more long. Efficiency was also reduced if the size of the tube was increased. For example, a 730-mm. length of 8.6-mm. inside diameter tubing had an absorption efficiency of 90 to 95% at 15 to 25 liters per minute. Presumably a longer length of this tubing was needed for complete absorption. Efficiency of absorption in the larger tubes was reduced a little by using 30- or 32-gage instead of 26-gage wire, probably because the smaller wire induced less turbulence in the gas stream. A tube of 7-mm. bore exhibited high efficiency but it could not be operated at rates above 10 liters per minute, whereas the 8.6-mm. bore tube would readily carry 25 liters per minute. The 7-mm. bore tube could carry 6 liters per minute when the liquid rate was 15 ml. per minute. Carrying capacity could be increased appreciably by adding 1% butyl alcohol to depress the surface tension of the solution, but the efficiency was not changed.

The absorber has also been used effectively for gas concentrations up to 8% by increasing the water flow and reducing the gas rate in 5 to 7-mm. bore tubes. Evidently almost any condition of absorption can be met by the proper choice of bore and length of tube.

An excess of hydrogen peroxide is essential. The stock solution is stable, but about 10% of the peroxide is catalytically decomposed by the platinum black in each cell, making an over-all decomposition of 30% on passing through the system, in addition to that used up by reaction with sulfur dioxide, although only one cell precedes the absorber. Solutions stronger than 0.004 M evolve an appreciable amount of oxygen in the cells, which may interfere somewhat with the conductivity measurements. For this reason, when it is necessary to use more peroxide, a flow cell, with dip electrodes, as shown in Figure 5, may be used.

A number of tests were made with different amounts of peroxide. When a fivefold excess was present, the efficiency of absorption was not appreciably increased by making the excess 15-fold. Even a slight excess seemed to be very effective. In an experiment with 15 p.p.m. of sulfur dioxide which used up nearly all the peroxide in a 0.002 M solution the efficiency of absorption was 93%, as compared with 96% in a 0.006 M solution. When the gas concentration was increased to 24 p.p.m., nearly all the excess sulfur dioxide beyond the capacity of the 0.002 M peroxide passed through the absorber, whereas the 0.006 M solution absorbed 95% of the gas.

RATE OF RESPONSE

Two factors need to be considered in determining the rate of response of the system to changes in the sulfur dioxide content of the air. The first is the lag due to the volume of liquid in the cell and in the U-tube ahead of the cell. This will depend on the length and bore of the tube, and the dimension of the cell. Obviously the recorder will lag behind any absorber by the time required to displace this solution. In addition, if the absorption takes place in some degree over the whole area of the absorber, full response of the recorder will be determined by the time required for complete displacement of the capillary liquid in the absorber.

A large number of empirical tests were made in which a sudden change of concentration was produced in the absorber, by alternately making connection at B, Figure 1, to a steady mixture of sulfur dioxide in air, or to a soda-lime tube. It required about 25 to 30 seconds before the recorder gave its initial response. This

presented displacement of the liquid in the U-tube. In addi-on, about 14 seconds were required to displace the 0.7 ml. in the ll. However, the absorber contained about 4 ml. of capillary ater which required more than a minute for displacement. It as noted that about 35 seconds after the recorder showed its itial response to the change of gas concentration, 50% of the ange was registered on the recorder. Similarly, 80% of the ange was registered in about 60 seconds; 90% in 90 seconds; id 95% in 120 seconds. Complete response usually required to 5 minutes. For practical purposes the record can therefore e considered to be a running average concentration of 1 to 2 inutes' duration—that is, being registered about 2 minutes ter the time of sampling. Smaller tubes and higher rates of quid flow will, of course, increase the rate of response.

"INSTANTANEOUS" ABSORBER

If more rapid and more detailed registration of a variable gas oncentration than can be obtained with the regular absorber in igure 1 is desired, a tube of the type illustrated as an alternative bsorber in Figure 1 may be used, in which rapidity of response is btained at the expense of efficiency of absorption.

It consists of two No. 26 gage platinized platinum wires, X, retched taut by the screws, Y in the center of a 10-mm. outside diameter tube, and spaced about 1 to 1.5 mm. The absorbing liquid is led onto the wires at E which then serve not only as the capillary path of the liquid but also as conductivity electrodes. Platinum wire has sufficient tensile strength to maintain a nearly permanent calibration, but platinum-clad steel wire strung on a steel frame to avoid undue strain on the glass would be a preferable arrangement. The absorbing liquid forms a continuous capillary film between the two wires if the liquid flow exceeds a minimum value (1.5 ml. per minute for 750-mm. wire length). The response of this absorber to a change of gas concentration is nearly as rapid as a micromax recorder can follow. With a liquid flow of 3.3 ml. per minute, recorder response is nearly complete in 5 to 10 seconds, and 10 to 15 seconds suffice for complete response. Efficiency of absorption is 20 to 40%, depending primarily on the gas rate. This absorber requires empirical calibration with known gas concentrations.

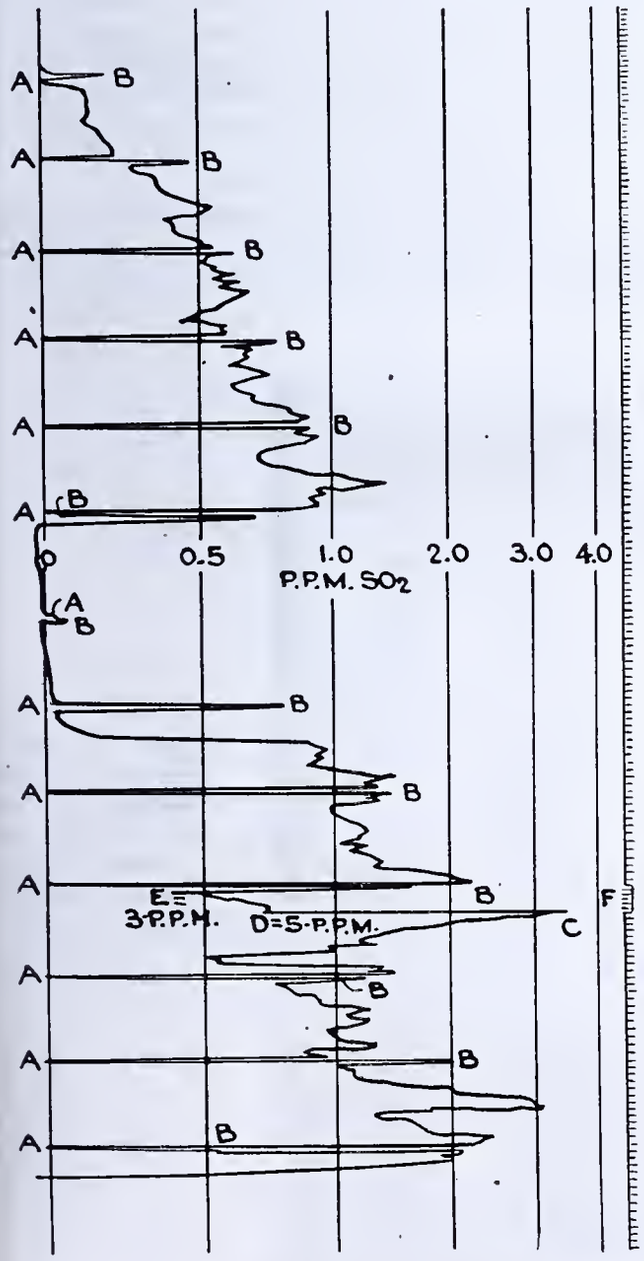

Figure 3. Type of Record Obtained with Field Autometer

APPLICATIONS

Several units of the equipment have been operated in the field, and it is evident that the new method can generally replace, to advantage, the older "accumulation" method for field sulfur dioxide analyses. Figure 3 illustrates the type of record that may be obtained.

Points A give the initial conductivity of the absorbing solution, displaced a little for ease of identification. Points B give the conductivity of the accumulated solution at 50-minute intervals. Between points A and B the flow cell is registering, indicating considerable fluctuation of the concentration. It may be noted that nearly every point B falls about where one would expect the average value of the preceding short-time readings to occur.

At point C, the concentration threatened to go beyond the range of the recorder. The latter accordingly operated a latching relay switch which disconnected the longer portion of the divided plate in the conductivity cell (Figure 1). The registration fell back to D and continued to rise, then fell off to E, when the accumulating cell was switched in. If the registration had fallen off a few more divisions beyond E, the recorder would have reconnected the plate in the conductivity cell. This actually occurred during the change from B to A. Definite proof that the recorder operated in the high concentration range is furnished by the track which registered gas volume along the right-hand edge of the chart. At F, the volume pen moved from right to left. When the low concentration range was registered, the pen moved from left to right.

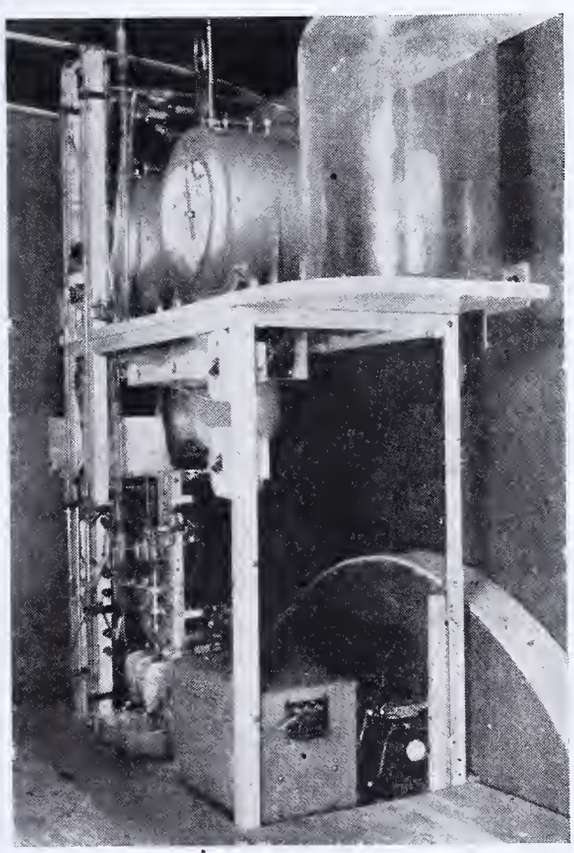

Figure 4. Double Autometer with Facilities for Igniting One Sample. Equipment Mounted in Truck

The new absorber also works satisfactorily with other soluble gases like ammonia (*4*) and hydrogen chloride, but it is ineffective with carbon dioxide. This is to be expected because of the relative solubilities of these gases (*10*). However, the optimum absorber dimensions for ammonia and hydrochloric acid have not been worked out.

Because accurately timed mechanical valves are not used, the new system is easily adapted to the multiple analyses previously described (*9*) for sulfur dioxide, hydrogen sulfide, organic sulfur, hydrogen chloride, and organic chlorine. A double unit has been constructed similar to Figure 2, in which one of the air samples may be ignited. Two clock-switching mechanisms are provided, one of which alternates every minute the connection between the two flow cells and the recorder; the other causes the registration of the two "accumulation" cells and the "feed" cell during 4 minutes of each half hour. Figure 4 is a photograph of this machine mounted in an insulated truck for ready transportability. The instrument can be used only where a source of power is available.

Another machine has been built at the suggestion of E. P. Fleming to control the operation of a pilot plant making elemental sulfur. This plant burns sulfur dioxide from metallurgical processes with sufficient natural gas to combine with all the oxygen, both in the sulfur dioxide and in the free state. The furnace is operated at about 1200° C., and hydrogen sulfide and sulfur dioxide are the principal reaction products. The desired ratio of these gases is two volumes of hydrogen sulfide to one of sulfur dioxide. The latter then react in a catalyst chamber to give sulfur and water vapor.

Figure 5 is a double photograph of the analyzer as seen from two directions.

Measured samples of gas from the plant containing 0.5 to 3.0% total sulfur are drawn through the system by two slow-moving piston pumps provided with mercury check valves, while a third smaller pump adds a definite proportion of oxygen to the samples. The gas is filtered to remove sulfur. The hydrogen sulfide in one of the samples is burned to sulfur dioxide by contact with a faintly glowing platinum coil before absorption. The hydrogen sulfide in the other sample passes through its absorber unchanged. Practically complete absorption of sulfur dioxide is obtained in a 350-mm. length of 5-mm. bore tubing, using a liquid flow of 6 ml. and a gas flow of 100 ml. per minute. Drying bottles and soda-lime tubes are provided following the absorbers to protect the mercury check valves and the pumps. The conductivity flow cells are connected as two arms of a Leeds & Northrup recording Wheatstone bridge. Any departure from a predetermined ratio of the conductivities of the two solutions then causes a valve on the natural gas supply to open or close, thus modifying the reaction in the furnace in the desired direction. With a variable sulfur dioxide supply, this machine and a modification of it, employing larger rates of gas and liquid flow, have played an important role in the successful operation of the plant.

Finally, a simple compact apparatus has been constructed, using Zenith gear pumps, for the analysis of high concentrations (up to 10% or more) of sulfur dioxide, which is comparable in speed, accuracy, and simplicity to the conventional "hot wire" method, but is not affected by admixture of carbon dioxide.

DISCUSSION

It is well established that at the interface between a liquid and a gas there are thin layers of the two substances with physical properties different from those in the body of either liquid or gas. These layers have been referred to as "stationary films" (*5*), and absorption of a solute by a liquid from a gas is largely determined by the manner in which the solute molecules traverse these films.

Table I gives the values for the initial rate of absorption of sulfur dioxide under a number of conditions, as found by several investigators. The results are calculated to a comparable basis and are expressed as grams of sulfur dioxide absorbed per square centimeter per minute from a gas mixture containing 2 p.p.m. (volume). The rate in water decreases, owing to the vapor pressure of the solute as the absorption proceeds, so that only the initial rate is significant for this comparison.

Rate of absorption is increased on a quiet surface, owing to stirring the liquid below the surface, and is increased still more when alkali is added with subsurface stirring. Wetted walls are evidently more efficient than quiet surfaces, probably because of

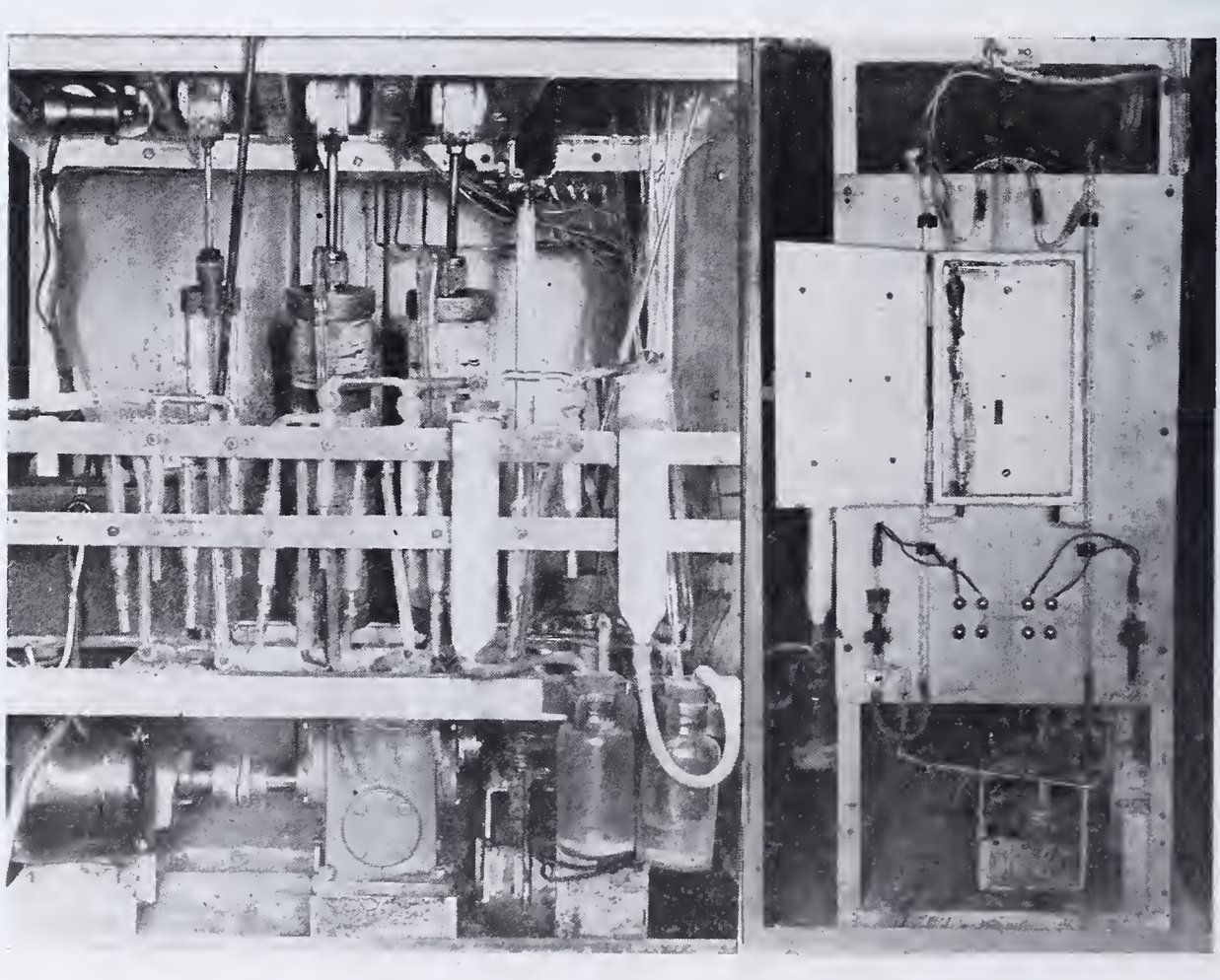

Figure 5. Analyzer Designed to Control Operation of a Sulfur Plant by Maintaining a Constant Ratio of Hydrogen Sulfide to Sulfur Dioxide

Table I. Initial Rates of Absorption of Sulfur Dioxide

Partial Pressure, tmosphere	Liquid	Surface	Absorption Rate[a]	Observer
1.0	Water, not stirred	Quiet	0.10	Becker (*1*)
0.03	Water, stirred	Quiet	0.15	Whitman and Davis (*10*)
0.5	1.8 *M* KOH, stirred	Quiet	0.63	Davis and Crandall (*2*)
0.1	Water flowing	Wetted wall tower[b]	1.2	Haslam *et al.* (*3*)
2×10^{-6}	0.002 *M* H_2O_2	Wetted wall tube[c]	3.5	This paper

[a] Grams of SO_2 per sq. cm. per min. $\times 10^7$, calculated to a partial pressure 2×10^{-6} atmosphere in each case.
[b] Flow: gas, 121 liters per min.; liquid, 1800 cc. per min. in 76-mm. be (10° C.).
[c] Flow: gas, 15 liters per min.; liquid, 3.3 cc. per min. in 7.6-mm. tube 5° C.).

ıe continuous formation of new films on the former. The ab-ɔrption rate in the 7.6-mm. tube is three times more rapid and ıe ratio of gas to liquid flow 65-fold greater than in the 76-mm. ɔwer. This difference in rate is explained by the presence of ydrogen peroxide in the small tube and is in accord with the ob-ervation that the latter is a poor absorber after all the peroxide is sed up. The behavior of this tube suggests that most of the quid is present in an absorbing film, since efficiency is affected only slightly by varying the excess of peroxide. The average film thickness is about 0.02 cm.

No data could be found in the literature giving the initial absorption rate of sulfur dioxide from bubbles. Carbon dioxide (*2*) is absorbed 70 times as rapidly from a bubble surface as from a subsurface-stirred quiet surface. If sulfur dioxide behaves similarly, the estimated rate of absorption from bubbles would be about 3 times the value given for the new absorber in Table I. This would explain the high efficiency of the simple bubbler previously described (*8*).

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A Photographic-Viscometric Apparatus and Technique

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ın apparatus and procedure for the accurate determination of flow mes of dilute solutions in the Ostwald-type viscometer have been eveloped. Means of measurement and control of the viscometric ariables have been designed on the principle of making approxi-ıately equal contributions to the final error. A unique feature of ıe apparatus is the automatic timing device. Motion pictures are ıade of meniscus transits in the viscometer and of the virtual image f an accurate, electronically operated clock. Times of transit are ıterpolated from the pictures to 0.001 second. Corrected flow mes of three or four check runs for each of three cellulose acetate ɔlutions in dioxane ranging from 0.01 to 0.3% concentration fall ıithin a time range of 0.005%.

MPROVEMENT of the accuracy of the measurement of the flow times of dilute high-polymer solutions in conjunction with uitable calibration or correction of the viscometer will provide iscosity data which will make possible a more critical evaluation f the several methods of extrapolation (*6*, *7*, *9*) for obtaining ntrinsic viscosities.

The realization of this end is, of course, based on a proper ppreciation of the variables involved (*1*, *4*, *5*, *10*, *13*) and the levelopment of apparatus embodying the inferred degrees of ɔrecision in the measurement or control of these variables. An ɔutstanding treatment of this problem has already been pro-ided by Jones and Talley (*8*). Perhaps the most notable feature ɔf their work was a photoelectric timing device which auto-natically recorded flow times to a precision of 0.01 second. In he present work, a photographic timing device is used, which ɔossesses several advantages not inherent in the photoelectric iming device. The entire timing record of a run, including dentification, is contained on about 0.5 meter of 16-mm. film. The times of transit of the initial and final marks on the viscom-ter may be obtained by interpolation from six to twenty pre- and ɔost-transit readings. The standard deviation of reading the transit time of any run may be made subject to calculation by statistical means.

ANALYSIS OF VISCOMETRIC ERROR

Design of the apparatus was based on an analysis of error with the intent that error contributions from the several variables controlled and measured would be of the same magnitude. To achieve a given coefficient of variation $\frac{s}{\bar{x}}$ for an individual result the permissible error contributions of the variables may be calculated from the equation

$$\frac{s}{\bar{x}} = \sqrt{\left(\frac{s_x}{\bar{x}_x}\right)^2 + \left(\frac{s_y}{\bar{x}_y}\right)^2 + \left(\frac{s_z}{\bar{x}_z}\right)^2 + \cdots}$$

The primary variables, considering the case of the Ostwald viscometer, are three: time, temperature, and pressure. The volume of flow and the dimensions of the capillary are constant. Pressure may be analyzed into three subordinate variables: sample volume, sample density, and viscometer alignment. To obtain a coefficient of variation for this five-variable condition of 0.01% the contribution of each variable should be 0.004%. An aim of 0.003% was set for the measurement or control of each of the five variables to provide a reasonable margin to cover small oversights or the failure quite to meet the specification on some one variable. The required magnitudes are shown in Table I, calculated for an assumed 100-second flow time in an Ostwald viscometer of conventional proportions.

Table I. Permissible Error Contributions of Variables

Variable	Corresponding to ±0.003%
Time	±0.003 second per 100 seconds
Temperature	±0.002° C. for dioxane at 25° C.
Sample volume	±0.001 ml.
Sample density	±0.3 mg. for 10-gram pycnometer sample
Viscometer alignment	±30 seconds of arc in plane of limbs of viscometer

Drainage and surface tension errors, which affect volume of flow and pressure, are best limited through proper design of the viscometer (*3*). They are not given explicit consideration in this analysis. The effect such errors would have on the relative viscosities of dilute solutions in a single solvent can be assumed to be substantially less than other contributions to error.

The components in the apparatus are described in the same order as the variables to which they relate. A schematic diagram of the assembled components of the apparatus is shown in Figure 1.

PHOTOGRAPHIC TIME RECORDER

An electrical 60-cycle frequency standard, *A*, accurate to ±0.001% provides the basis of time measure. (The frequency standard was specially made by American Time Products, Inc. It has an output at 115 volts of 40 watts.) Current from this frequency standard is used to operate 60-cycle, 115-volt synchronous motors which drive a 25-cm. (10-inch) dial, sweep second clock, and a 5-inch dial conventional electric clock, *B*. (The 10-inch dial sweep second clock consumes 5 watts alternating current. It is made by the Standard Electric Time Company.) This timing combination is calibrated by audio-visual comparison to standard time signals broadcast by WWV, the National Bureau of Standards radio station, and received on radio *W*. Error of 0.001% corresponds to 0.3 second in 8 hours, a readily observable difference.

The 5-inch dial clock is mounted in front of and concentric to the 10-inch dial clock by means of three fine wires (see Figure 2 for detail). The clocks are so placed that a spherical mirror, *C*, establishes a virtual image of the clocks in juxtaposition to the measuring bulb of an Ostwald-type viscometer, *D*, immersed in a thermostatically controlled water bath, *L*. A 16-mm. motion picture camera, *E*, operated at 32 frames per second, is focused to photograph the virtual image of the clocks and the measuring bulb of the viscometer. The clocks operate continuously. The exposure lever of the camera is manually operated during the transits by the meniscus of the two graduations of the viscometer. (Functional relations of camera, clocks, mirror, and viscometer are shown in Figure 3.) To "stop" the second hand of the clock a 0.001-second exposure time is required. It was obtained by using a disk shutter with a 12° opening in place of the shutter with a 167½° opening supplied with the camera. Lighting demands, using 80 Weston (tungsten) film and an *f*1.9 lens, are moderate.

The necessarily rapid passage of the upper graduation of the Ostwald viscometer by the meniscus is secured by reduction of the internal diameter of the upper tube to about 1 mm. Small diameters permit spanning and resultant bubble formation in the upper tube.

THERMOSTAT

The thermostatically controlled water bath is simple in construction but can be made to operate with a variation as small as ±0.001° C.

The water bath, *L*, is constructed of copper, lagged with cork and contains a 0.6-cm. (0.25-inch) plate glass front. The capacity is 120 liters. Circulation is provided by means of an Aminco centrifugal pump-type stirrer. The pattern of flow is stabilized by means of the glass vane, *T*. Flow from the pump is directed on the cooling coil, *O*, and heating coil, *P*, which are grouped together. A highly sensitive Aminco mercury-in-glass thermoregulator is placed directly in the wake of the heating and cooling coils. This reduces to a minimum the lag in the heating cooling cycle. The rate of heating of the 200-watt heating coil is controlled by the variable transformer, *S*. The average heat consumption is about 50 watts. An adjustable constant rate of cooling is obtained by use of a constant-level device, *Z*, on the cooling water supply. The optimum adjustment requires that the rate of cooling, heater off, be slow, about 0.006° C. per minute, and that the rate of heating, heater on, be approximately equal. Under these conditions thermal lag in the thermoregulator is reduced to a minimum. The critical element is the thermoregulator. It must have a well-defined cycle of on-off operation within 0.001° C. If these conditions are met, the temperature control indicated will result. The true temperature of the bath was determined by reference to a thermometer with corrections certified to 0.01° by the National Bureau of Standards.

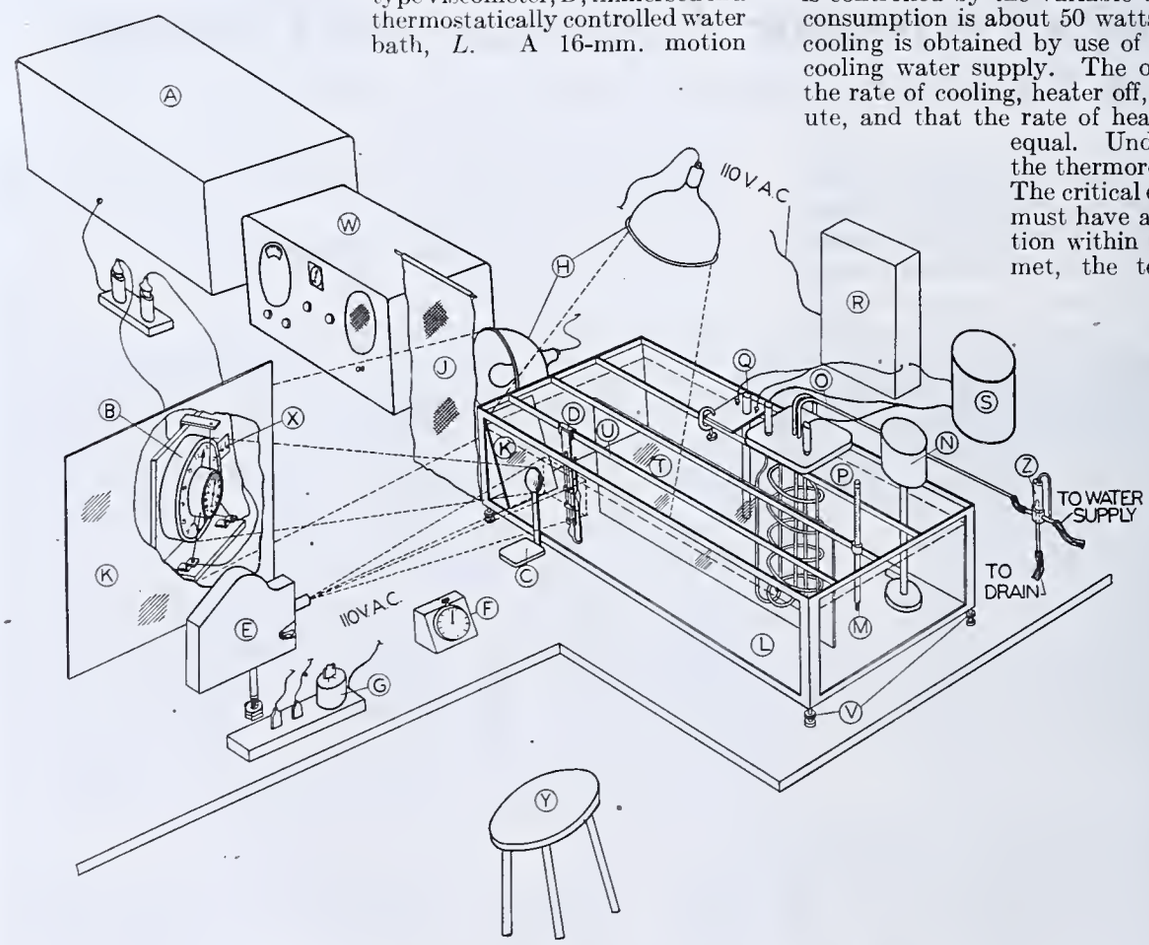

Figure 1. Schematic Diagram of Photographic Viscometer

A. Standard 60-cycle, 115-volt source
B. Clock assembly
C. Spherical, surface-silvered mirror
D. Viscometer and clamp
E. 16-mm. motion picture camera
F. Stop watch for rough checks
G. Switch for photographic illumination
H. Photoflood lamps and reflectors
J. Cheesecloth diffusion screen
K. Opal glass diffusion plates
L. Thermostat bath
M. Beckmann thermometer
N. Circulating pump
O. Cooling coil
P. Heating coil
Q. Sensitive thermoregulator
R. Electronic relay
S. Variable transformer for regulation of heating rate
T. Glass vane for directing circulation
U. Level, lapped brass bar for supporting viscometer clamps
V. Leveling screws
W. Short-wave radio receiver
X. Run identification tag
Y. Operator's position
Z. Constant-level water supply for cooling coil

SAMPLE VOLUME AND DENSITY

Analytical Balance Pipet, Pycnometer. The sample volume is roughly determined by a pipet, the accurately calculated from the density and the weight increase of the loaded viscometer determined on an analytical balance. The loaded viscometer is also weighed after the viscosity determination. The time rate of change in charge volume due to volatile loss is calculated. From this rate the average solvent loss per run is obtained and a corrected volume calculated for each trial.

The sample density is determined in a 10-ml. Ostwald-type pycnometer thermostatically controlled to ±0.001° C. and weighed to 0.1 mg.

VISCOMETER CLAMP, LEVEL, AND VISCOMETER

A viscometer holder consisting of a screw clamp, two brass -blocks, and a spring clamp, all securely attached to a stainless eel column, D, is used. The heavy brass bar, U, upon which is mounted is secured to the thermostat frame. The vertical ignment of the clamp is checked by means of a highly sensitive vel, 30 seconds of arc per 0.1 inch, mounted perpendicular to a od, which is held as the viscometer is held. Adjustment in level made by turning the bolts, V (Figure 1) which function as legs r the thermostat. (Figure 4 shows the viscometer clamp in etail and illustrates the use of the level in the alignment test.)

The viscometer in present use is the Ostwald type, capillary ngth, 12.5 cm., diameter 0.5 mm. Bulb capacity is approxiately 3 ml. and the charge volume about 6 ml. Diameter of the pper capillary is about 1 mm.

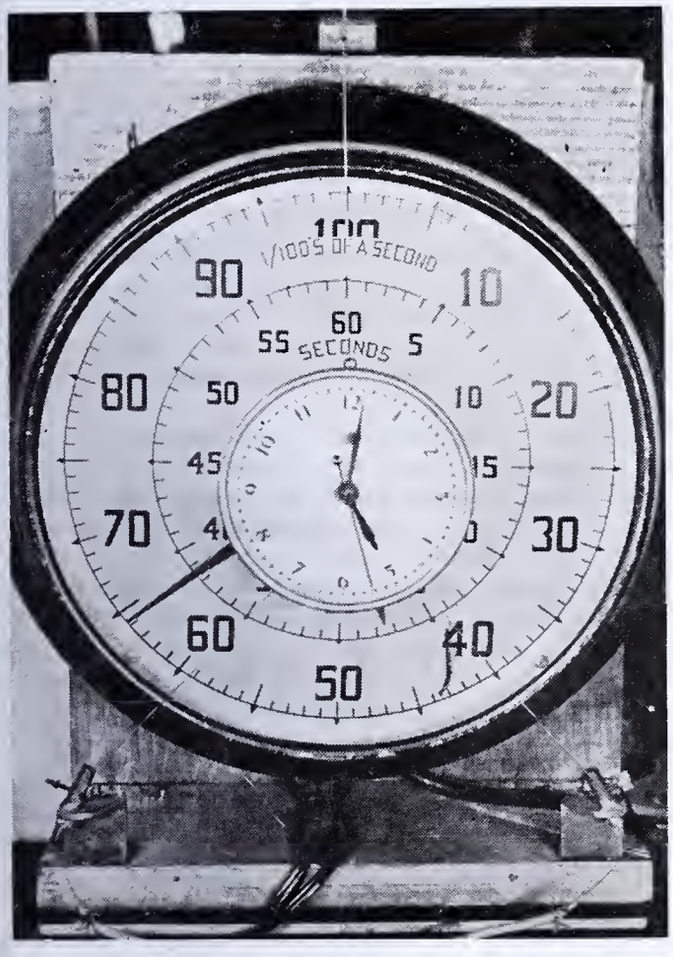

Figure 2. Clock Assembly

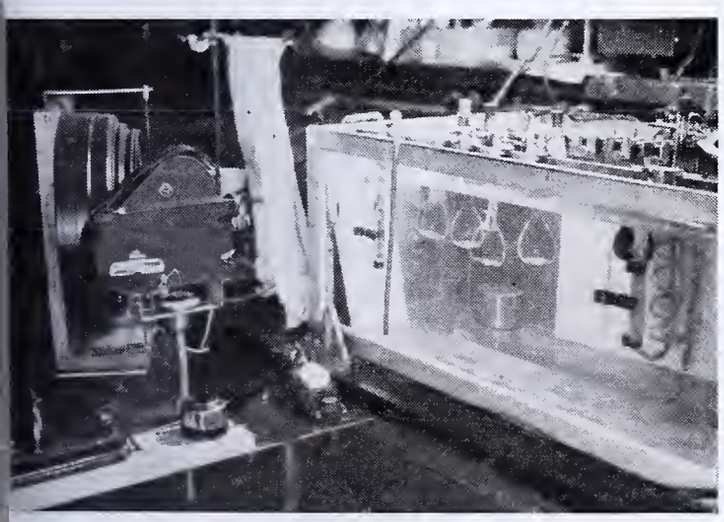

Figure 3. Functional Positions of Clocks, Mirror, Viscometer and Camera

Any viscometer that can be made to photograph satisfactorily can be used. A Ubbelohde viscometer would eliminate the sample volume considerations. Minor modifications would adapt the apparatus for use with the Hoeppler or other falling ball viscometers.

PROCEDURE

The most necessary single element in a flow time determination is the avoidance of dust particles in the viscometer. All cleaning fluids entering the viscometer pass through a fine sintered plate funnel (Figure 5). The viscometer is dried with air drawn through the same sintered plate. Cleaning fluids and air are propelled through the viscometer by attachment of the effluent side to a vessel maintained at reduced pressure, B'.

Once cleaned and dried, the viscometer is stoppered with dust-free rubber stoppers and weighed. The sampling pipet is cleaned and dried similarly.

The sample is drawn into the pipet through a fine sintered plate filter stick (Figure 5). A flash evacuation of the pipet, by proper manipulation of stopcock A', suffices to draw in the sample. Concentration changes caused by volatile loss may be shown by calculation to be negligible.

The filtered sample is transferred from the pipet to the weighed viscometer, and is shaken to assure air saturation and to put any dust which may be present in motion so that it may be seen. The presence of visible particles disqualifies a sample. If the liquid appears dust-free, the sample weight is obtained.

The viscometer, equipped with inverted tubes at both openings to keep out dust, is placed in the clamp in the thermostat and let stand for 10 minutes. The sample is then drawn up slowly by an aspirator bulb to above the top graduation. Clocks and viscometer are illuminated by operation of switch G (Figure 1). The sample is let flow and the transit of the upper graduation by the meniscus is photographed. Photographic illumination is immediately turned off. It is turned on again to photograph the

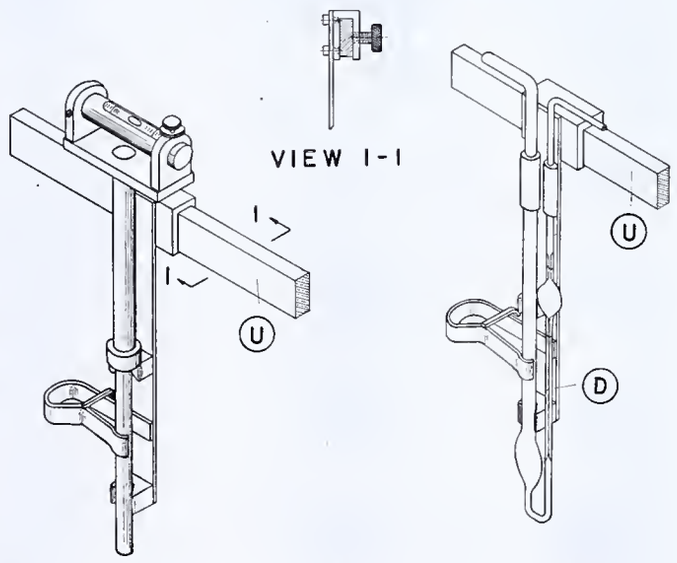

Figure 4. Sensitive Level, Clamp, and Viscometer

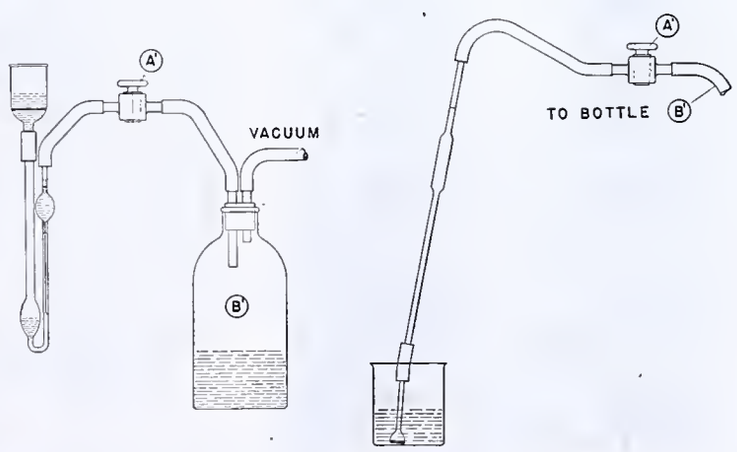

Figure 5. Cleaning Device for Viscometer and Pipet

Table II. Flow Times of Cellulose Acetate Solutions in Dioxane

Mark	Concn.	Observed Time	Observed Temp.	Sample Volume	Correction Factors: Temp.[a]	Correction Factors: Sample volume[b]	Correction Factors: Density[c]	Corrected Time
	% w/w	Sec.	° C.	Ml.				Sec.
64	0.009707	366.299	24.994	5.9846	0.999894	1.000152	1.000040	366.331
		366.308	24.994	5.9840	0.999894	1.000169	1.000040	366.346
		366.315	24.992	5.9834	0.999859	1.000187	1.000040	366.347
		366.308	24.992	5.9828	0.999859	1.000203	1.000040	366.345
55	0.09749	425.832	25.001	5.9987	1.000018	0.999758	1.000292	425.861
		425.806	25.002	5.9981	1.000035	0.999773	1.000292	425.848
		425.809	25.001	5.9975	1.000018	0.999791	1.000292	425.851
62	0.2934	589.728	24.997	5.9869	0.999947	1.000090	1.000808	590.227
		589.711	24.996	5.9863	0.999930	1.000110	1.000808	590.211
		589.690	24.996	5.9857	0.999930	1.000133	1.000808	590.204

[a] Based on temperature coefficient of viscosity for dioxane of $\frac{1.76\%}{°\,C.}$.

[b] Read from calibration curve relating flow time to sample volume for dioxane. Factor is unity for sample volume of 5.9900 ml.

[c] Solution density referred to density of dioxane solvent of 1.02781 grams per ml. at 25.00° C.

transit of the bottom graduation. (If illumination is left on for more than several seconds, the bath temperature is measurably increased.) Temperature during the trial is recorded. At least three checks are made. The viscometer is removed from the thermostat and dried and the final sample weight obtained.

The negative film is developed and projected on a microfilm reader. A plot of the distance of the meniscus from the graduation versus time may be used to give, by interpolation, the time of transit to an apparent ±0.001 second. Time of flow is the difference in upper and lower transit times. A photographic record of a single run and a plot of the data read from it are shown in Figures 6 and 7. As the relation of meniscus position to time is linear, an analytical expression may instead be used to treat the data from which the time of transit and the standard deviation of a reading may be calculated.

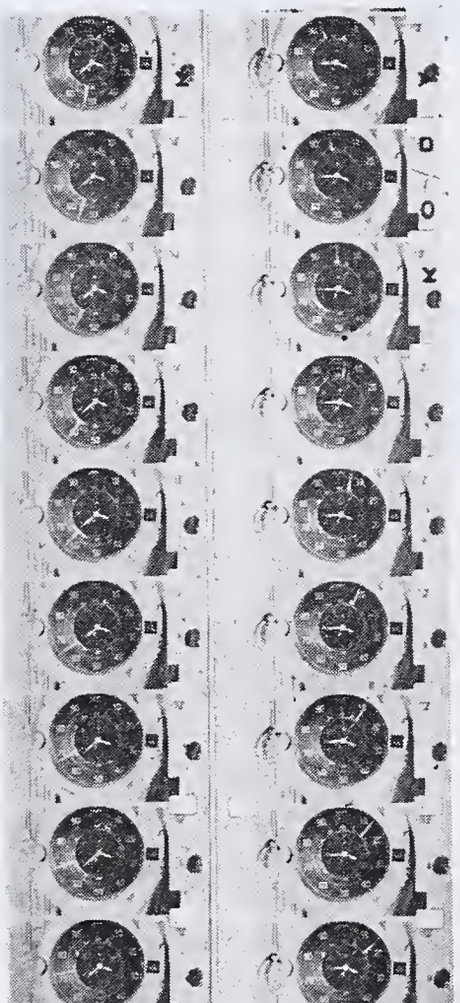

Figure 6. Photographic Timing Record

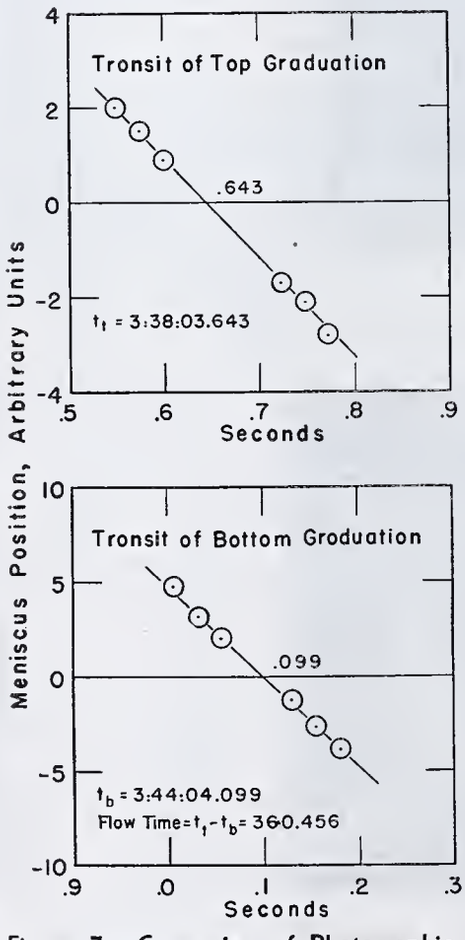

Figure 7. Conversion of Photographic Timing Data to Transit Times

TREATMENT OF DATA

A corrected flow time is calculated by correcting the charge volume to a standard value, correcting for the effects of density on driving force, and for temperature deviation, if any.

Results obtained for a commercial cellulose acetate dissolved in 1,4-dioxane and the factors for converting to corrected flow times are shown in Table II.

In each instance the time for the first trial is farthest from the average, suggesting temperature equilibrium had not been quite attained. Even including first trials, each set has a range of less than 0.005%, inferring a standard deviation of the order of magnitude sought.

The corrected times shown in the last column will, of course, require the application of a calibration or kinetic energy correction before being used in the calculation of relative viscosities. Procedures for determining and applying the kinetic energy correction are reported elsewhere (*2*, *5*, *11*, *12*, *14*).

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Electron Diffraction and Electron Microscope Study of Oxide Films Formed on Metals and Alloys at Moderate Temperatures

Stripped Oxide Films of Metals

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The oxidation process occurring on metals has been studied by electron diffraction and electron microscopy, using oxide films stripped by procedures suggested by Evans and co-workers. The apparatus and techniques are briefly described. The metals studied include chromium, cobalt, copper, iron, molybdenum, nickel, aluminum, columbium, and tungsten. The electron micrographs and electron diffraction patterns are presented and discussed. The oxide films are shown to consist of small oxide crystals ranging in size from 100 to 2500Å.

THE physical and chemical structure of the oxide films formed on metals and alloys is of considerable interest in our understanding of their protective properties. The authors (*10, 14*) have studied the structure of the oxide films formed on metals and alloys by the reflection method of electron diffraction. These studies show that chemical and physical transformations occur during the formation and heating and cooling of the oxide film. Inasmuch as the reflection method samples only the outer surface of the film, the information obtained is incomplete. The bulk structure and composition may be considerably different from that on the outer surface. In addition, the nature and size of the crystals in the oxide film can only be approximated by the reflection technique.

This paper presents electron microscope and electron diffraction evidence concerning the structure of electrochemically and chemically stripped films from a series of nine metals which had been oxidized under known conditions: iron, nickel, cobalt, chromium, molybdenum, tungsten, columbium, aluminum, and copper.

ELECTRON MICROSCOPE TECHNIQUE

The use of the electron microscope for the study of the submicroscopic fine structure of matter is well known (*1*). Commercial instruments are available with a resolving power of less than 40 Å. Thus, the shape of particles and the nature of the mosaic structure of a system of crystals may be approximated for particles of 150 Å. or larger. The low penetrating power of 60-kv. electrons in matter limits its direct use to oxide films of the order of 500 Å. in thickness. The preparation of such films requires the use of electrochemical or chemical methods for stripping the oxide film from the metal.

SURVEY OF THE LITERATURE

STRIPPING OF FILM. The presence of a film on passive iron was proved by Evans (*4*) who stripped films too thin to show interference colors from metals.

His first method was based on electrochemical action, using the oxidized metal as the anode in order to dissolve the metal underlying the film. This method was applied to oxidized electrolytic iron, copper, and aluminum to secure films which were examined with the light microscope. The second method was based on direct chemical attack of the underlying metal by a saturated solution of iodine in 10% potassium iodide.

Later Evans and Stockdale (*5*) extended and modified the electrochemical method to remove oxide films from iron, copper, nickel, carbon steel, and stainless steel. For removal of the film from iron, saturated potassium chloride in a hydrogen atmosphere was used as the electrolyte. Hydrogen prevented contamination of the removed film by secondary reaction products caused by oxidation of the dissolved iron.

Vernon, Wormwell, and Nurse (*23*) modified Evans' iodine method by using the reagent developed by Rooney and Stapleton (*19*) to remove oxide films from iron and carbon steel. In this technique both oxygen and water were eliminated as possible contaminating agents by employing a solution of iodine in anhydrous methyl alcohol in a nitrogen atmosphere.

Several chemical methods have been successful in removing films from different metals. Withey and Millar (*26*) developed an analytical method of estimating the oxide in or on aluminum which has been applied by several workers to the removal of the oxide film. Aluminum metal is removed from its oxide film by treatment with hydrogen chloride at 300° to 400° C.; the oxide film is retained intact. Wernick (*24*) and Fischer and Kurtz (*6*) removed oxide film from aluminum by scratching the film and immersing in saturated mercuric chloride solutions. Kutzelnigg (*16*) dissolved tin foil away from its oxide by a 10% ferric chloride solution. Tammann and Arntz (*22*) rendered visible the film present on silver by placing on the surface a drop of mercury, which spreads below the film, raising it from the metal.

TRANSMISSION ELECTRON DIFFRACTION OF OXIDE FILMS. Most of the oxide films on massive pieces of metal have been studied by the reflection method.

Darbyshire (*3*) studied stripped oxide films of nickel and copper by the transmission method; the patterns were identified as NiO and Cu_2O, respectively.

Oxide films of iron, nickel, and copper were stripped from the metals and examined by Smith (*20*). Air-heated, first-order interference colored films of iron gave patterns corresponding to Fe_3O_4 or γ-Fe_2O_3. Heating of one of the films to 600° C. gave a pattern which resembled more nearly that of γ-Fe_2O_3 as prepared by dehydration of γ-FeOOH.

Iitaka, Miyake, and Iimori (*15*) claimed that the film stripped from iron passivated in dichromate was γ-Fe_2O_3, not Fe_3O_4.

Steinheil (*21*) obtained transmission patterns of aluminum oxides after stripping from the metal. At room temperature the oxide formed was a face-centered cubic structure with A_0 = 5.35 Å. When the oxide was formed by heating in a small flame it conformed to the x-ray structure of γ-Al_2O_3.

White and Germer (*25*) measured the rate of oxidation of thin copper films by transmission of both the copper and cuprous oxide.

ELECTRON MICROSCOPY OF OXIDE FILMS. Henneberg (*13*) showed micrographs of the oxide films stripped from iron, aluminum, and nickel.

Mahl (*17*) obtained micrographs of stripped oxide films of electrolytic iron and aluminum. The iron oxide films showed a granular structure, while those of aluminum showed both granular and amorphous structures, depending on the treatment of the metal.

The micrographs by Fischer and Kurtz (*6*) of stripped aluminum oxide films show a definite granular structure. The films were prepared by electrolytic oxidation by either direct or alternating current and by using sulfuric or oxalic acid. The film was stripped from the metal by the mercuric chloride technique.

APPARATUS

ELECTRON DIFFRACTION CAMERA WITH FURNACE. This apparatus is described in previous papers (*8, 10, 14*). The polished metal sample is oxidized under controlled conditions of tem-

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perature, time, and oxygen pressure in the electron diffraction furnace. The progress of the oxidation is studied in situ by electron diffraction at the temperature of the reaction.

Electrolytic Apparatus for Removing Oxide Films. Two modified versions of the apparatus described by Evans (*5*) are used to remove the oxide film from the metal by electrolysis.

The apparatus shown in Figure 1 consists essentially of three parts, cell *A* for electrolysis in an hydrogen atmosphere, reservoir *B* with oxygen-free wash water, and hydrogen purifying parts *C* and *G*.

A has three side arms, fitted with stopcocks. T_2 leads directly to the source of purified hydrogen. Electrolytic tank hydrogen is purified by passing the gas over copper turnings at 300° C. and through a solution of sodium bicarbonate in *C*. Hydrogen pressure is used to force the wash water from *B* through T_3 into cell *A*. The electrolyte and washings are removed by T_2. The cell is divided into two compartments by a fritted-glass filter.

Boiled, saturated potassium chloride solution is placed in *A*. Purified hydrogen from *C* is bubbled alternately through the solutions in both arms by a tube (not shown in Figure 1) leading directly from *C* into the cell at the openings at the top. After this preliminary blow-out of the air, the purified hydrogen enters T_2 to sweep out the air above the solution continuously during the electrolysis. The oxidized metal, *D*, is installed as the anode and is held in place by the rubber stopper, *F*, which has a small vent for escape of hydrogen. The cathode, *E*, is the same metal as *D*, or platinum.

The source of current is 110 volts direct current. The current passed through the cell is controlled by a potentiometer-rheostat and a second rheostat in series with the electrodes. The electrolysis is started at a current of 10 ma. and increased gradually to 60 ma. if the film is not loosened after one hour. The time necessary to remove the film varies from 0.5 to 1.5 hours.

As the electrolysis proceeds the loosened film detaches itself or is freed by lightly tapping the electrode. The film is recovered as squares 1 mm. on an edge because the oxidized surface had been ruled previously in such a manner. Washing of the pieces of film is accomplished by draining through T_1 and forcing wash water through T_3 with T_2 closed. After washing, the cell is disconnected at T_2 and T_3 to invert and to remove the pieces of film to a small dish.

Electron Microscope. A type EMB-4 microscope, equipped with an electron diffraction unit, is used.

CALIBRATION

Electron Diffraction. The use of the electron diffraction adapter of the electron microscope has been described in detail by Picard (*18*). Magnesium oxide is used as a diffraction standard. In the calibration procedure, the diameters, *D*, of the transmission diffraction rings are recorded. The lattice spacings, d_{hkl}, are obtained from x-ray diffraction tables (*11*). The calibration constant, Dxd_{hkl}, is calculated for the several rings, and the value is found to be 27.3. From the measured diameters of the unknown diffraction pattern, the values of the lattice spacings, d_{hkl}, are readily obtained.

Electron Microscope. The magnification is determined by establishing a relationship between the lower magnification range of the electron microscope with optical measurements on the same object. A 10.67-micron tungsten wire is used as the object. Having established this relationship, the higher magnifications can be calibrated by comparison of the distance between two particles at the high magnification with the same distance at a lower magnification where the relationship with the light instrument has been established. Dispersed titanium dioxide particles are used in this study.

PREPARATION OF SPECIMENS

The specimens are machined from bars of the metals to cylinders 0.92 cm. (0.375 inch) in diameter and 0.375 inch long, and after cleaning, are heat-treated at elevated temperatures in dissociated ammonia gas or in wet hydrogen. The details of the heat treatment and subsequent metallographic polish are shown in Table I. The specimens are stored in a desiccator over anhydrous calcium chloride.

The metal specimen is placed in the electron diffraction camera furnace (*8*, *10*, *14*) and heated to the proper temperature. Oxygen to a pressure of 0.1 atmosphere is admitted and the specimen is oxidized for a predetermined time. The time and temperature

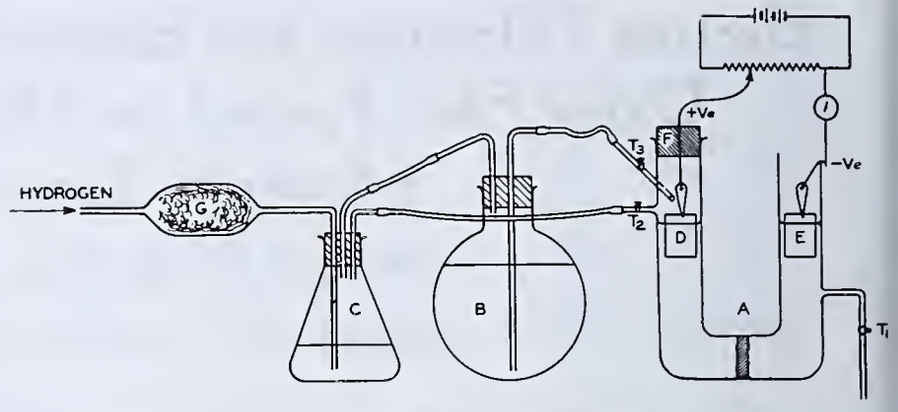


Figure 1. Electrolytic Apparatus for Removing Oxide Films

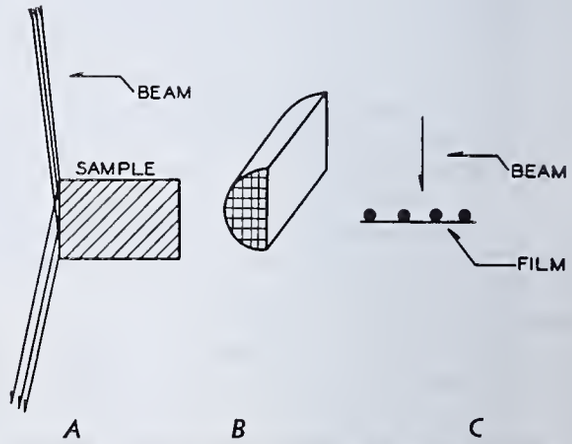


Figure 2. Specimen before and after Electrolysis

A. Reflection diffraction
B. Film on metal
C. Film mounted for electron microscopy

Table I. Analysis and Preparation of Metal Specimens

Metal	Analysis	Heat Treatment	Polishing
Cr	Electroplated on polished oxygen-free high conductivity Cu	None	Emery paper No. 1, 320 wax wheel, chrome rouge, No. 1 alumina
Co	Electrolytic P 0.003, Al 0.033, Al_2O_3 0.004, C 0.0015	1000° C. for 15 hours in dissociated ammonia gas	Emery paper No. 1, 320 wax wheel, chrome rouge, No. 3 alumina
Cu	Oxygen-free, high conductivity	900° C. for 15 hours in dissociated ammonia gas	Emery papers through 000, chrome rouge, No. 3. alumina
Fe	Research Puron, 99.96%	1000° C. for 15 hours in wet H_2	Emery papers through 000, chrome rouge, No. 3 alumina with 95% alcohol
Mo	99.95+%	1200° C. for 1 hour in dissociated ammonia gas	Emery papers through 000 chrome rouge, Nos. 1 and 3 alumina
Ni	Commercial grade, C 0.02, Mn 0.29, Si 0.02, Cu 0.07, Co 0.80, Fe 0.15	1000° C. for 12 hours in dissociated ammonia gas	Emery paper No. 1, 320 wax wheel, chrome rouge, No. 3 alumina
Al	99.986%	None	Emery papers through 000, chrome rouge, Nos. 1 and 3 alumina
Cb	99.9+%	None	Emery papers through 000, chrome rouge, Nos. 1 and 3 alumina
W	99.9+%	10 hours at 1200° C. dry H_2	Emery paper through 000, chrome rouge, Nos. 1 and 3 alumina

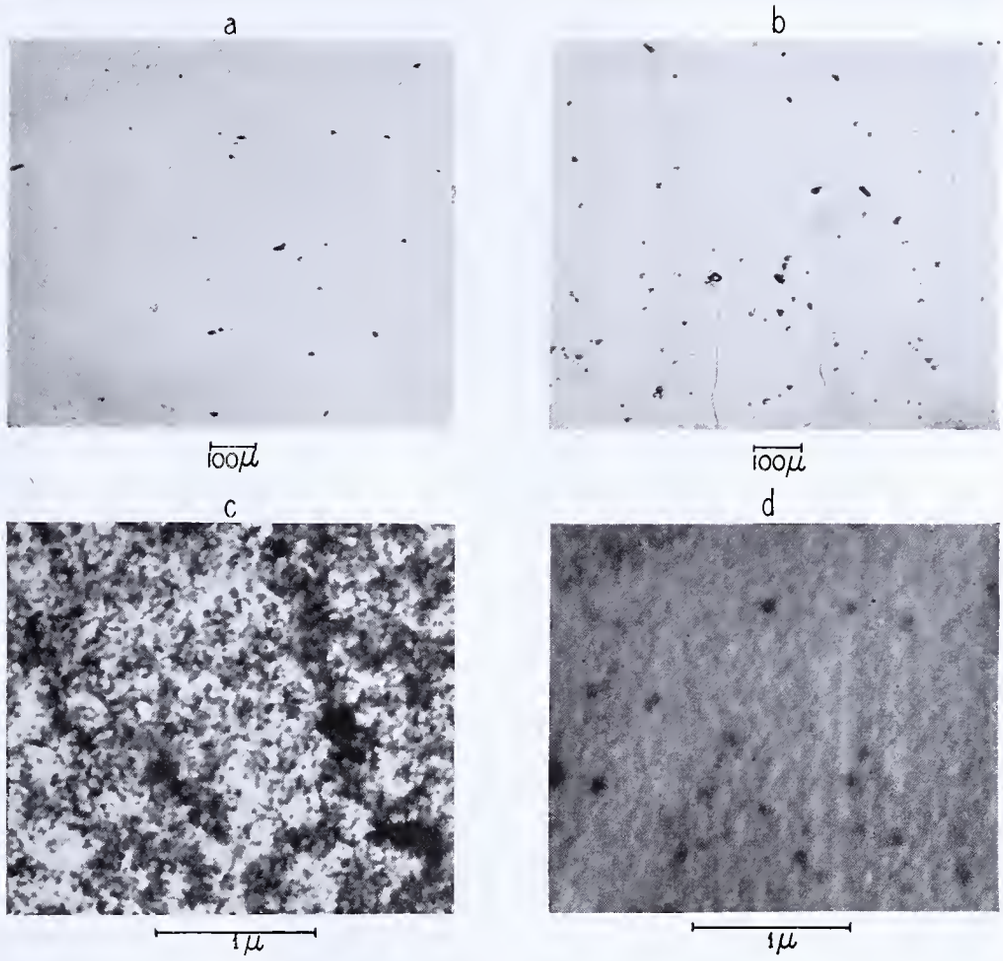


Figure 3. Study of Oxidation of Nickel

a. Light micrograph, polished
b. Light micrograph, oxidized
c. Electron micrograph, stripped oxide film
d. Electron micrograph, replica, oxidized surface

conditions of the oxidation are estimated from rate measurements where available. Oxide film thicknesses of the order of 1.0 to 3.0 micrograms per sq. cm. weight of oxygen can be studied effectively. Where rate measurements are not available, it is necessary to make intelligent guesses as to the oxidation conditions.

Electron diffraction reflection patterns are taken of the sample after oxidation and after cooling in vacuo to room temperature. The specimen is removed and cut carefully in two portions; one portion is used for making photomicrographs at 100 and 1000×.

A thin film of Parlodion is formed on the surface of the other portion by placing a small drop of 0.01% solution of Parlodion in amyl acetate on the surface and draining off the excess solution. After the film is dry, the surface is scratched with a sharp point of a needle into 1-sq. mm. sections. To protect the sides and bottom of the sample from electrochemical or chemical action, these parts are painted with a protective coating. The details of this part of the sample preparation are shown in Figure 2.

The sample is now placed either in the electrolytic cell shown in Figure 1 or in a beaker for chemical attack for removal of the film. Chemical or electrochemical attack occurs at the scratches where the metal is exposed. As the attack develops the film is gradually loosened from the metal. The films are washed by hot oxygen-free distilled water in the electrochemical cell or in the beaker. The small squares are now ready for mounting on the specimen screens and are placed in the microscope for study after drying in a vacuum chamber at room temperature.

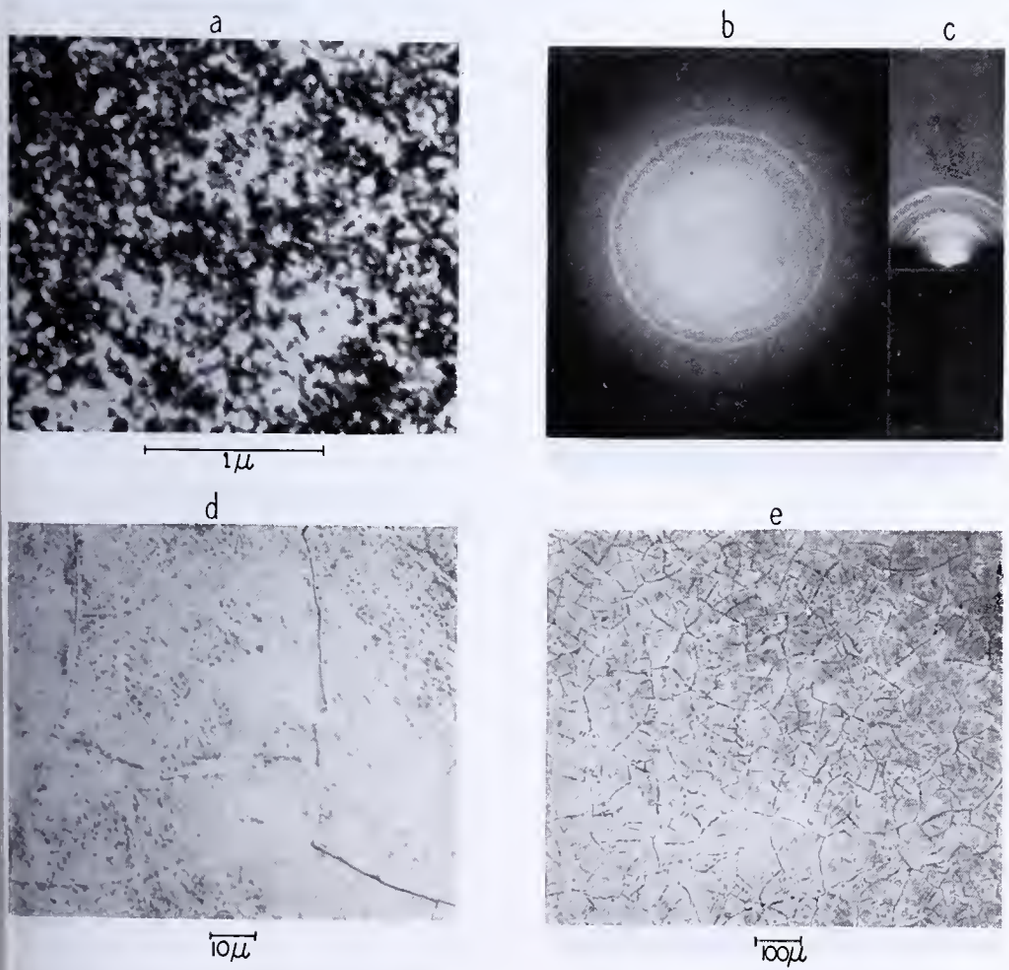


Figure 4. Oxide Film of Chromium, Cr 5–600

a. Electron micrograph stripped film
b. Electron diffraction transmission stripped film
c. Electron diffraction reflection film on metal
d, e. Light micrograph film on metal

METHOD OF INTERPRETING DATA

Electron Diffraction. The electron diffraction reflection method has been discussed in previous papers (*8*, *10*, *14*). The methods used in the interpretation of data by the transmission technique are similar to those used for the reflection method. In the transmission technique the specimen is the sample of stripped film. The diffraction patterns obtained are a series of concentric rings instead of half circles as in the reflection method.

The transmission method possesses several advantages over the reflection method. The diffraction rings are sharper and the background of incoherent scattering is less. The transmission method also yields information on the structure of the whole film, while the reflection method indicates only the structure of the outer surface. This is important, since the surface structure is probably different from the structure of the body on the film.

Table II. Electron Diffraction Data

(Oxide films formed on the metals at 0.1 atmosphere of O_2 at various times at various temperatures)

Metal	Temp. ° C.	Time of Oxidation Min.	Diffraction Patterns: Preoxidation by R[a]	Oxidized by R	Oxidized at 25° C. by R	Stripped by T
Cr	600	5	Cr_2O_3, VD[a]	Cr_2O_3, S	Cr_2O_3, S	Cr_2O_3, M
	600	30	Cr_2O_3, VDO	Cr_2O_3, SO	Cr_2O_3, SO	Cr_2O_3, D
Co	200	50	None	CoO, 4.28[a], VD	CoO, 4.28, VD	CoO, 4.24; Co_3O_4, 8.07, D
	300	10	Co_3O_4, 8.12, VD	Co_3O_4, 8.12, SO	Co_3O_4, 8.12, DO	CoO, 4.25; Co_3O_4, 8.06, S
	400	5	Co; CoO, 4.28, VDO	Co; CoO, 4.28, SO	Co; CoO, 4.28, SO	CoO, 4.24, MO
	400	10	CoO, 4.22, VD	CoO, 4.22, S	CoO, 4.22, S	CoO, 4.23, M
	400	30	CoO, 4.28, VDO	CoO, 4.28, S	CoO, 4.28, S	CoO, 4.24, MO
	500	5	CoO, 4.29, VDO	CoO, 4.29, S	CoO, 4.29, S	CoO, 4.24, MO
Cu	200	5	Cu_2O, 4.32; CuO, VD	Cu_2O, 4.32; CuO, S	Cu_2O, 4.32; CuO, S	Cu_2O, 4.23, MO
	200	30	Cu_2O, 4.33, VD	Cu_2O, 4.33, S	Cu_2O, 4.33, S	Cu_2O, 4.24, DO
Fe	250	5	Fe_3O_4, 8.49, DO	Fe_3O_4, 8.49, DO	Fe_3O_4, 8.49, DO	Fe_3O_4, 8.35, M
	250	30	Fe, VD	Fe_3O_4, 8.44; α-Fe_2O_3, SO	Fe_3O_4, 8.44; α-Fe_2O_3, SO	Fe_3O_4, 8.35; α-Fe_2O_3, SO
	300	5	Fe_3O_4, VD	Fe_3O_4, 8.43; S	Fe_3O_4, 8.43, S	Fe_3O_4, 8.37, DO
Ni	400	20	NiO, 4.20, VD	NiO, 4.20, S	NiO, 4.20, S	NiO, 4.17, SO
	450	5	NiO, 4.20, VDO	NiO, 4.20, MO	NiO, 4.20, MO	NiO, 4.15, DO
	500	5	NiO, 4.20, VD	NiO, 4.20, MO	NiO, 4.20, MO	NiO, 4.15, SO
	500	20	NiO, 4.22, VD	NiO, 4.22, MO	NiO, 4.22, MO	NiO, 4.15, MO
	500	60	....	....	....	NiO, 4.17, DO
	550	5	....	....	....	NiO, 4.19, SO
Mo	450	5	None	Mo_2O_3, S	Mo_2O_3, S	Mo_2O_3, M
	500	5	None	Mo_2O_3, MoO_3, S	Mo_2O_3, MoO_3, S	Mo_2O_3, M
Al	500	5	None	α-Al_2O_3 γ-Al_2O_3, VD	α-Al_2O_3 γ-Al_2O_3, VD	γ-Al_2O_3; α-Al_2O_3 VD
Cb	400	5	None	CbO, M	CbO, M	Cb_2O_5; CbO, SO
W	400	5	None	None	None	WO_3, D
	450	5	None	None	None	WO_3, D

[a] Key: R, reflection; T, transmission; S, sharp lines; M, medium lines; VD, very diffuse lines; D, diffuse lines; O, oriented pattern. Numbers refer to unit cell size.

Electron Microscope. The electron microscope can be applied to the study of surfaces of opaque bodies in two ways: (1) If the structure of the surface is of interest, a polystyrene-silica replica may be made. Thus, if a thin oxide film is present on the surface, the replica will show the outer physical contours of the oxide film. A comparison of a micrograph of this replica with one made before oxidation would be interesting. (2) By use of the stripped film technique the details of crystals making up the body of the oxide can be studied.

To compare the two methods a specimen of polished nickel is oxidized at 400° C. for 20 minutes in 0.1 atmosphere of oxygen. The sample is divided into two parts. A polystyrene-silica replica is made of one half and the oxide film is stripped electrochemically from the other half. Figure 3 shows a comparison of the results achieved by the two methods. The replica shows more detail of the physical structure of the outer surface, while the stripped film shows more detail of the crystal structure of the oxide film. The stripped film technique is used in this study. Not only is more information obtained from the micrographs of the stripped film but the electron diffraction pictures of the body of the film may be compared with that taken of the surface by the reflection technique.

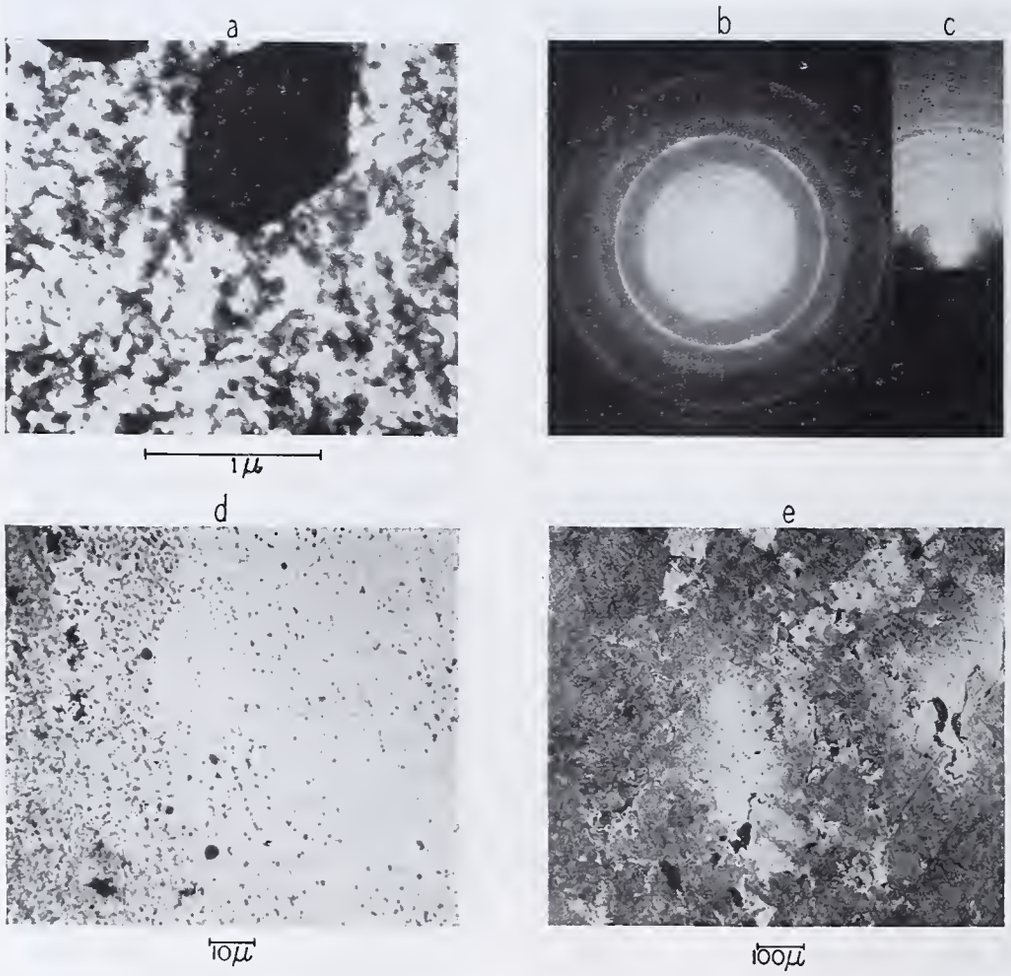


Figure 5. Oxide Film of Cobalt, Co 5–400

a. Electron micrograph stripped film
b. Electron diffraction transmission stripped film
c. Electron diffraction reflection film on metal
d, e. Light micrograph film on metal

Information Recorded. The following information is recorded from the electron micrographs of the stripped film: (1) particle size, (2) particle size distribution, (3) particle shape, (4) uniformity in film thickness, and (5) type of micrograph. The particle size is obtained by averaging measurements on a number of crystals, while the particle size distribution indicates the variation in particle size. The particle shape is determined from an examination of the more typical shapes in the pattern. Uniformity in film thickness refers to the presence of thick and thin portions of the film. The type of micrograph refers to a number of features of interest, including (1) the sharpness of the crystal edges, (2) the presence of overlapping crystals, and (3) the presence of extraneous material.

Table III. Electron Microscope Analyses of Stripped Oxide Films of Metals

Metal	Temp. °C.	Time of Oxidation Min.	Color of Film on Metal	Micrograph	Particle Size Å.	Size Distribution Å.	Shape	Uniformity	Type of Micrograph
Cr	600	5	Greenish yellow	4	400	250 to 750	Irregular	Fairly uniform	Medium sharp, overlapping crystals
	600	30	Mauve	[a]	400	250 to 750	Irregular	Nonuniform	Diffuse, overlapping crystals
Co	200	50	Light yellow	[a]	450	300 to 700	Irregular	Fairly uniform	Diffuse, overlapping crystals
	300	10	Mauve-blue	[a]	300	200 to 400	Irregular	Uniform	Medium sharp
	400	5	Blue and yellow	5	450	250 to 1000	Irregular	Fairly uniform	Sharp, overlapping crystals
	400	10	Pink and green	6	600	300 to 1500	Irregular and square, angular	Nonuniform	Sharp, overlapping crystals
	400	30	Greenish blue	[a]	600	300 to 1500	Irregular, angular	Nonuniform	Sharp, overlapping crystals
	500	5	Silver-pink II	[a]	1000	600 to 2000	Irregular, angular	Nonuniform	Sharp, overlapping crystals
Cu	200	5	Yellow	7	500	300 to 900	Irregular	Nonuniform	Medium sharp, overlapping crystals
	200	30	Brown	[a]	750	450 to 1500	Irregular	Nonuniform	Medium sharp, overlapping crystals
Fe	250	5	Light blue I	[a]	350	250 to 500	Irregular	Nonuniform	Diffuse
	250	30	Pink blue II	8	1200	500 to 1800	Irregular, angular	Fairly uniform	Sharp, overlapping crystals
	300	5	Silver-blue II	[a]	400	300 to 600	Irregular, angular, some large slender crystals	Nonuniform	Diffuse, overlapping crystals
Mo	450	5	Yellow-mauve I	9	500	250 to 1000	Irregular, angular	Nonuniform	Medium sharp
Ni	400	20	Yellow-mauve I	10	400	300 to 600	Irregular squares	Nonuniform	Sharp, overlapping crystals
	450	5	Silver-yellow	[a]	500	300 to 800	Irregular	Fairly uniform	Diffuse
	500	5	Yellow II	[a]	600	400 to 1000	Irregular	Uniform	Diffuse
	500	20	Yellow-red II	11	600	300 to 1500	Irregular	Nonuniform	Medium sharp
	500	60	Yellow-red II	[a]	600	300 to 1500	Irregular, angular	Nonuniform	Medium sharp
	550	5	Silver-yellow II	[a]	600	300 to 2000	Irregular	Nonuniform	Sharp, overlapping crystals
Al	500	5	Light yellow and metallic	12	500	100 to 2500	Large regular, small irregular	Nonuniform film discontinuous	Sharp, particles overlap
Cb	400	5	Dark blue and light purple	13	250	100 to 600	Very irregular	Nonuniform cracks	Medium, patchwork of thick and thin sections
W	400	5	Light reddish yellow	14	150	100 to 400	Round, irregular	Fairly uniform	Sharp, replica of metal grains

[a] Micrographs not shown.

These features are important in classifying a micrograph but are less definite in their interpretation.

RESULTS

Preliminary Experiments. The methods used for the stripping of oxide films from metals are well known, but several points concerning the general technique appear to need clarification. Are extraneous reaction products formed as a result of chemical or electrochemical action? Is it necessary to use a hydrogen atmosphere in the electrochemical cell? Does the stripping technique affect the chemical and physical structure of the oxide film?

Several stripping experiments have been made using both an air and a hydrogen atmosphere. Electron micrographs made from these films show more extraneous matter from the films stripped in an air atmosphere. A hydrogen atmosphere is adopted in the standard technique.

To study the effect of stripping on the chemical structure of the film, a specimen of Nichrome V is polished and then oxidized in the electron diffraction camera furnace. After cooling to room temperature, a reflection pattern is taken. The sample is now subjected to the stripping procedure. Before the film is completely loosened, the sample is removed, washed, and dried. It is placed in the electron diffraction camera and a reflection picture of the surface layer is again taken. No change in the chemical structure is noticed. This evidence, together with the fact that hydrated oxides of the metals have not been observed, indicates that the electrochemical stripping procedure does not affect the surface appreciably.

Oxide Films. In studying the oxide films formed on the metals at 0.1 atmosphere of oxygen for various times at various temperatures, the samples are heat-treated and polished as described in Table I before being placed in the electron diffraction camera furnace for oxidation. For most of the metals several oxidations are made under different time and temperature conditions. Results of electron diffraction study are shown in Table II.

Three reflection patterns and one transmission pattern are taken of the oxide film. The first reflection pattern is taken of the

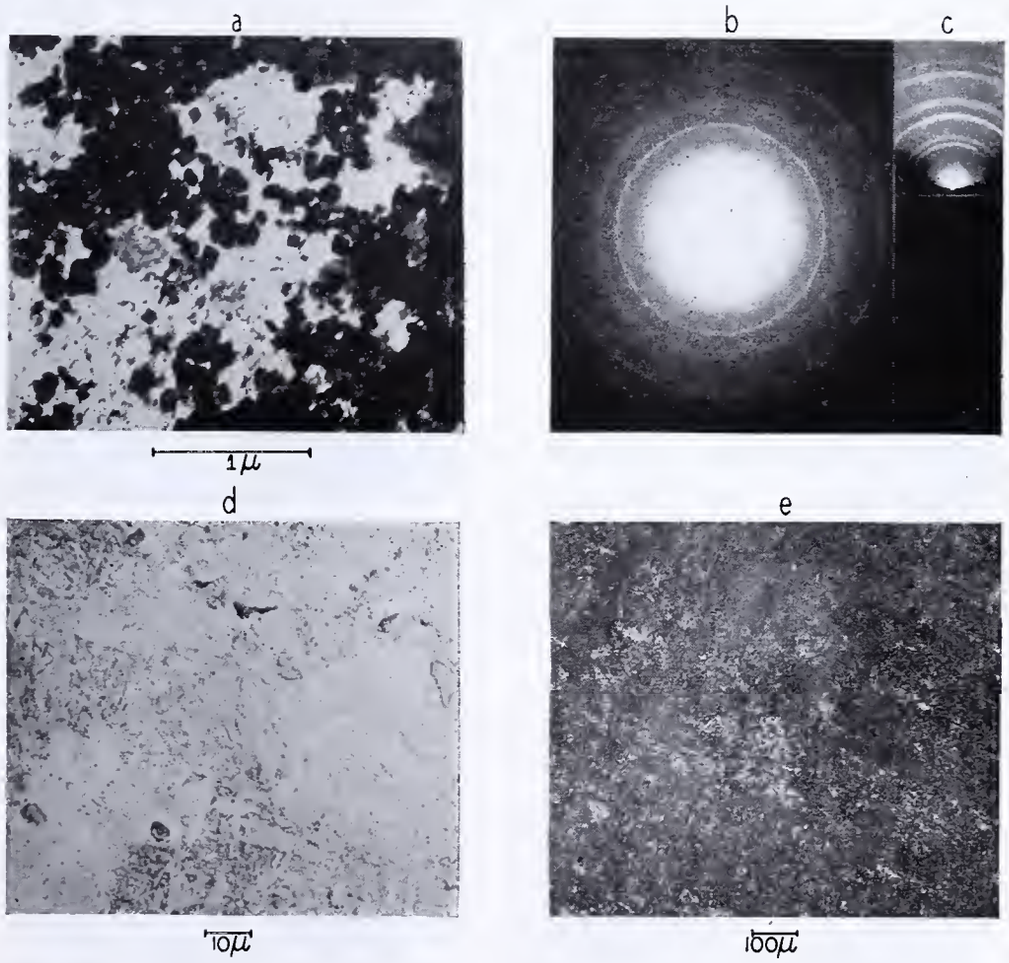


Figure 6. Oxide Film of Cobalt, Co 10–400

a. Electron micrograph stripped film
b. Electron diffraction transmission stripped film
c. Electron diffraction reflection film on metal
d, e. Light micrograph film on metal

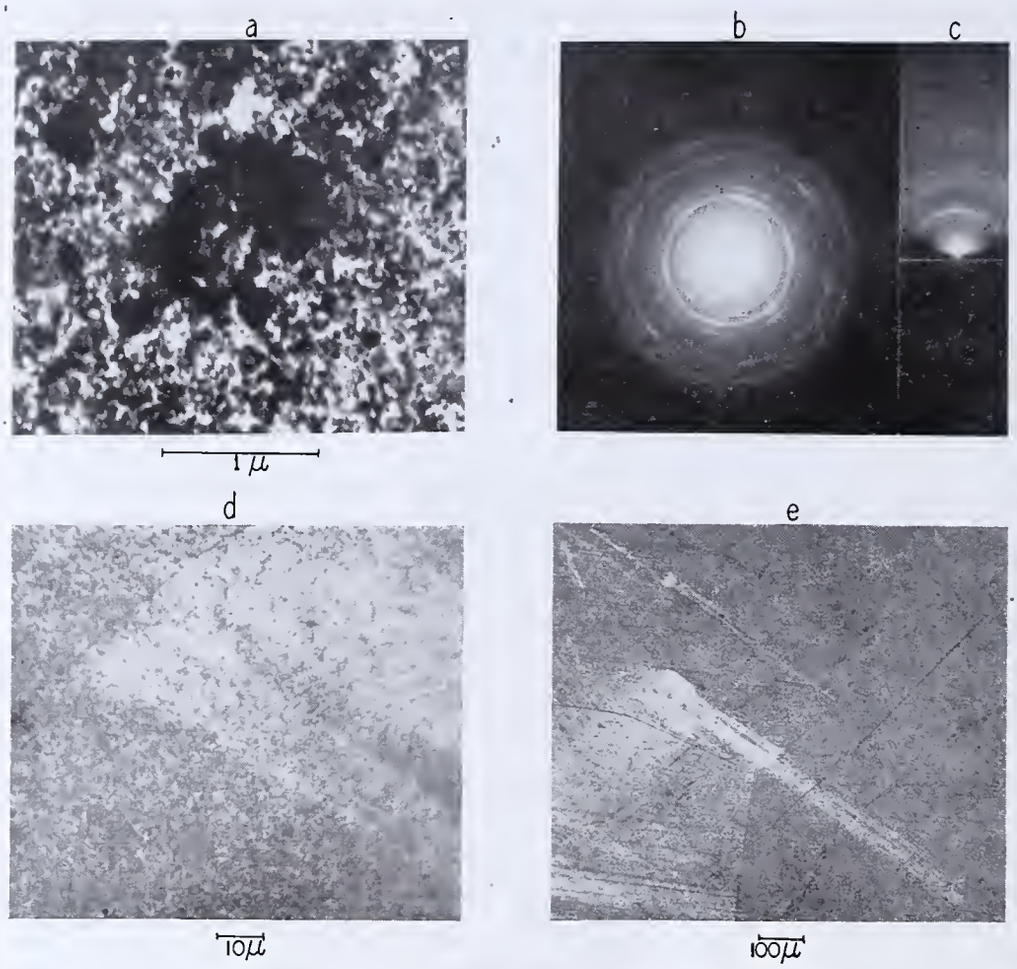


Figure 7. Oxide Film of Copper, Cu 5–200

a. Electron micrograph stripped film
b. Electron diffraction transmission stripped film
c. Electron diffraction reflection film on metal
d, *e*. Light micrograph film on metal

metal in the vacuum of the camera before oxidation and at the temperature of the experiment. The second is taken after the oxidation, and the third is taken after the oxidized sample is cooled to 25° C. in a vacuum. The transmission pattern of the stripped oxide film is also included, in order to compare the body structure of the film with its surface structure. Table II shows the chemical structure of the surface oxide, the unit cell size where readily calculable, and the type of diffraction pattern obtained.

Figures 4 to 14 show the light micrographs, electron micrographs, electron diffraction transmission patterns, and electron diffraction reflection patterns for the metal specimens. The lengths of 1, 10, or 100 microns are shown on the photographs. The light micrographs were taken of the unstripped oxide film at 100 and 1000×, while the electron micrographs were taken at 6800× and enlarged optically to 34,000×.

Table III summarizes the information recorded from the electron microscope: (1) color of oxide film on metal, (2) particle size in Ångströms, (3) particle size distribution, (4) particle shape, (5) film uniformity, and (6) type of micrograph.

DISCUSSION

Electron Diffraction. The experimental d_{hkl} values are compared to the values obtained from x-ray data and the patterns identified. The d_{hkl} values also are used to calculate the lattice parameters, a, for oxides of the cubic types. The x-ray data, used for identification purposes, are shown in Table IV. The lattice parameters for Mo_2O_3, CbO, and Cb_2O_5 have not been determined from the x-ray data.

Table V shows a comparison of the electron diffraction results obtained in this study with the x-ray parameters. The composition of the oxide films, with several exceptions in the cobalt and copper oxidation experiments, is shown to be similar by the transmission and the reflection methods. The oxides found can be correlated with known oxide structures. With the exception of several oxidation experiments on cobalt and copper, the oxides fit fairly well with the predictions of the time-temperature existence regions previously studied (*10*, *14*). Differences may be expected due to the polishing procedures employed and to the pressure influence on the existence chart.

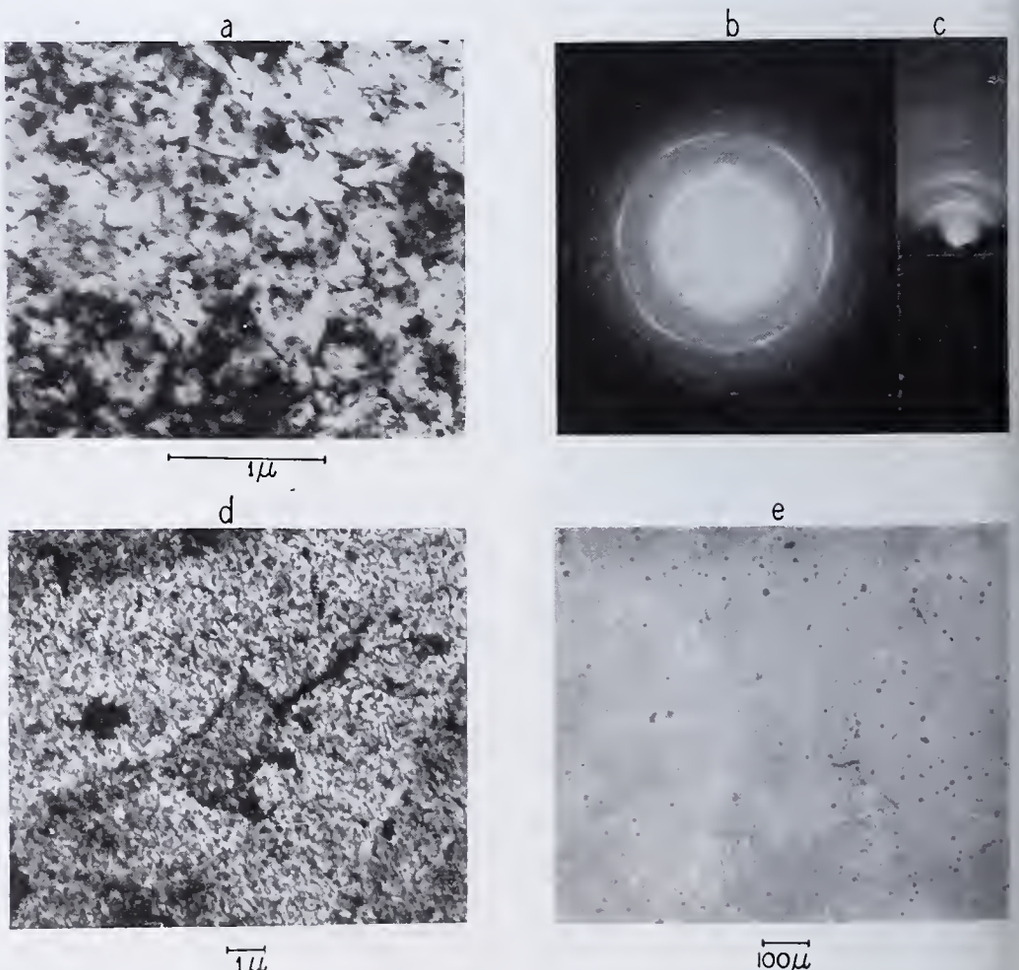


Figure 8. Oxide Film of Iron, Fe 30–250

a, *d*. Electron micrograph stripped film
b. Electron diffraction transmission stripped film
c. Electron diffraction reflection film on metal
e. Light micrograph film on metal

The lattice parameters of the oxide structures shown in Table V indicate deviations from the aeeepted x-ray data. A previous work (*10*) indicated that the electron diffraction refleetion method yielded lattiee paramcters slightly larger than the x-ray values. This positive deviation was attributed to either the presenee of metal in solid solution in the oxide lattiee or strains set up as a result of the formation of the oxide film.

Positive deviations are also notieed in the reflection measurements of this study. The transmission data show small negative deviations. A breakdown of the average deviations on a percentage basis of the oxide lattice parameters from the x-ray values is shown in Table VI. The precision involved in the measurement of the lattiee parameters is about 0.25% for the transmission patterns and about 0.4% for the reflection patterns.

The reflection method yields lattiee parameters which average 0.7% high, while the transmission method gives values which average 0.2% low. A cross calibration of the instruments was made by taking patterns of the same speeimen of stripped oxide film. The transmission electron diffraction pattern taken with the eleetron microscope gave an average deviation of d_{hkl} values from the x-ray values of less than 0.1%, while the electron diffraction high-temperature camera gavc an average deviation of 0.2%. This cross calibration indicated either that the positive deviations notieed on the reflection patterns are real or that some unknown faetor is affecting the reflection experiments. The improbability of relating this unknown factor to a variable specimen to plate distanee in the refleetion technique has been discussed (*10*).

Table IV. Oxide Lattice Parameters and Structural Type, X-Ray Data

Substance	Reference	Lattice Parameters a	b	c	α	Structural Type
FeO	*12*	4.28	..	..	...	Face-centered cubic
CoO	*12*	4.25	..	..	...	Face-centered cubic
NiO	*12*	4.17	..	..	...	Face-centered cubic
Cu_2O	*12*	4.24	..	..	...	Cubic
Fe_3O_4	*12*	8.40	..	..	...	Cubic spinel
γ-Fe_2O_3	*13*	8.32	..	..	...	Cubic spinel
Co_3O_4	*12*	8.11	..	..	...	Cubic spinel
γ-Al_2O_3	*13*	7.895	..	..	...	Cubic spinel
α-Fe_2O_3	*12*	5.42	..	..	55° 17′	Rhombohedral
Cr_2O_3	*12*	5.35	..	..	54° 58′	Rhombohedral
α-Al_2O_3	*12*	5.12	..	..	55° 17′	Rhombohedral
Mo_2O_3	*12*	..	..	..	...	.....
CuO	*12*	4.66	3.40	5.09	...	Monoclinic
WO_3	*12*	7.28	7.48	3.82	...	Monoclinic
CbO	*7*	..	..	..	...	.....
Cb_2O_5	*7*	..	..	..	...	.....

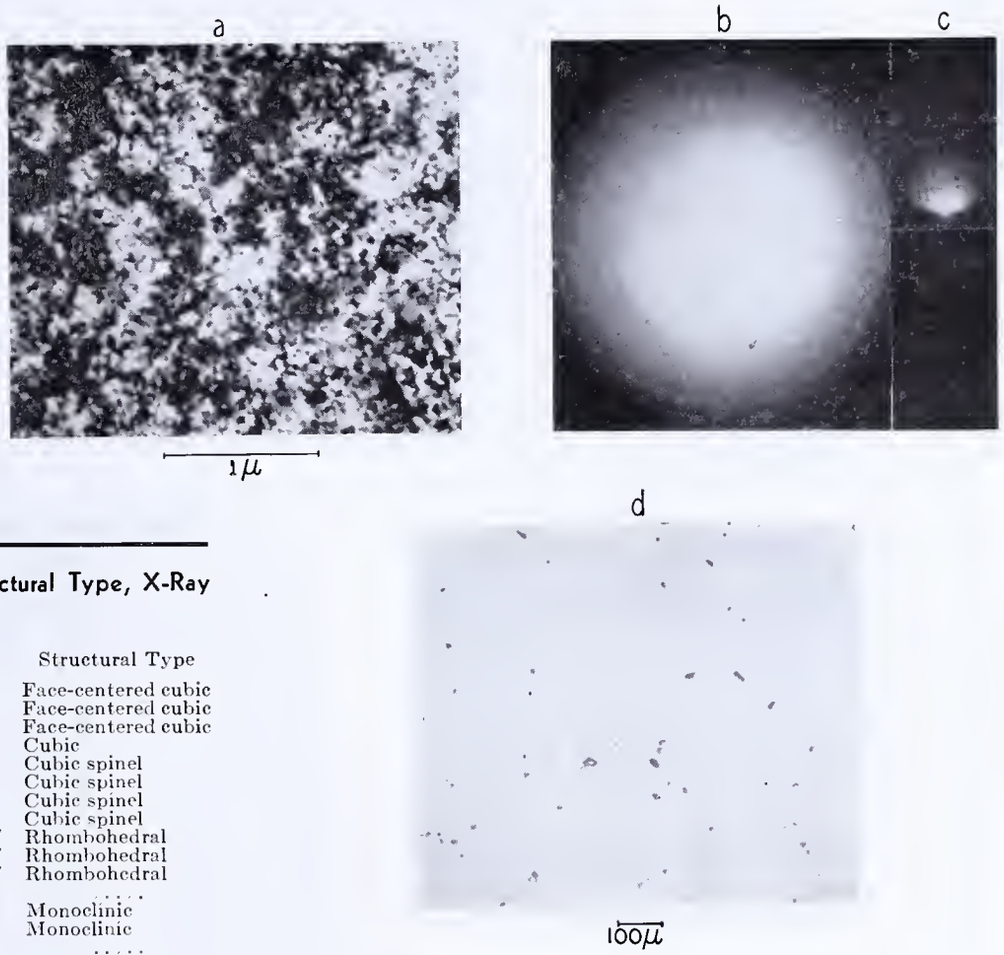
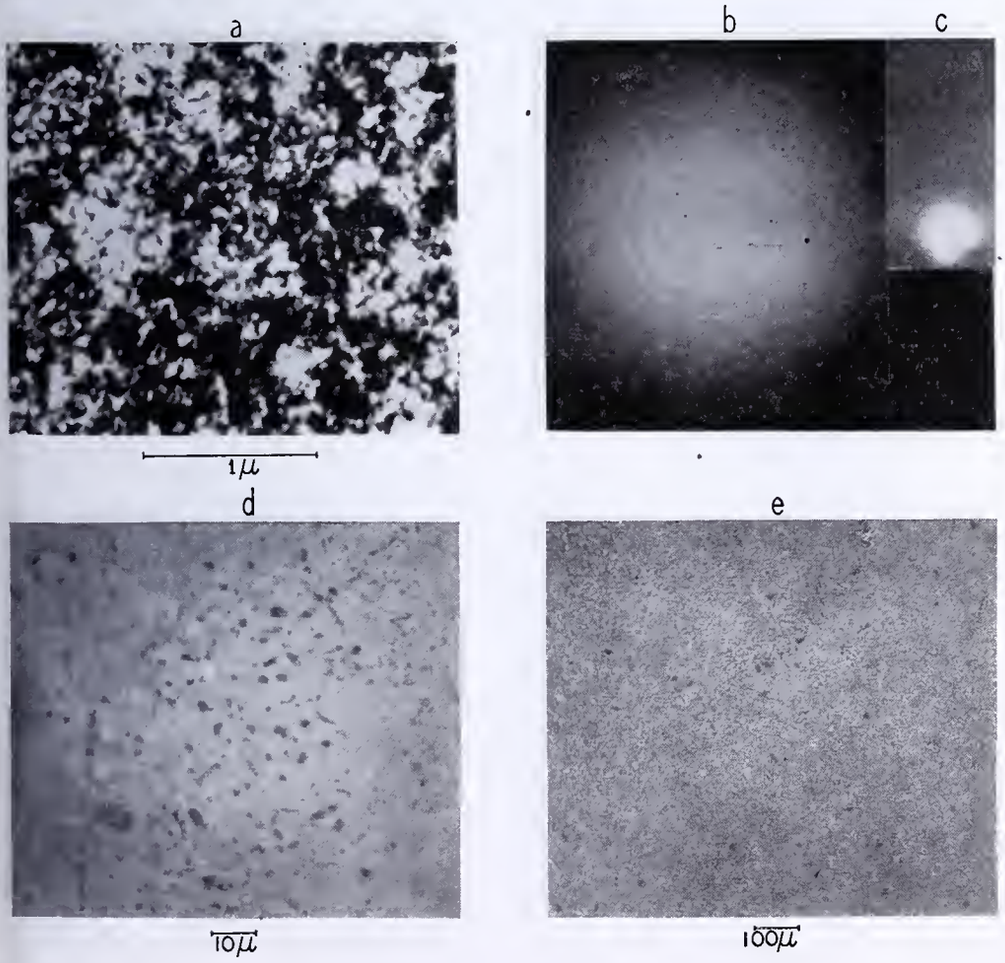


Figure 10. Oxide Film of Nickel, Ni 20–400

a. Electron micrograph stripped film
b. Electron diffraction transmission stripped film
c. Electron diffraction reflection film on metal
d. Light micrograph film on metal

Figure 9 (*Below*). Oxide Film of Molybdenum, Mo 5–450

a. Electron micrograph stripped film
b. Electron diffraction transmission stripped film
c. Electron diffraction reflection film on metal
d, *e*. Light micrograph film on metal



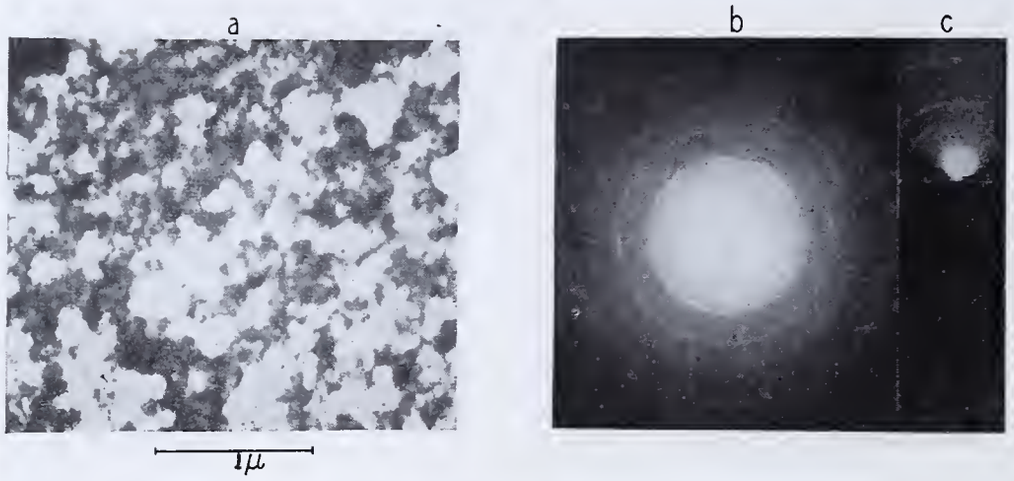


Figure 11. Oxide Film of Nickel, Ni 20–500

a. Electron micrograph stripped film
b. Electron diffraction transmission stripped film
c. Electron diffraction reflection film on metal
d. Light micrograph film on metal

rate of nucleation and the rate of growth of the oxide crystals. If the surface is limited in area and the rate of nucleation and growth sufficiently high, a two-dimensional mosaic structure will be formed. Further growth of a particular crystal must be at the expense of other crystals in the same plane or in a direction normal to the surface. The rates of nucleation and of growth are rate processes and are exponential functions of the free energy of activation and the temperature. These free energies of activation have unique values for each oxide and for each particular crystal

Table V. Lattice Parameters of Oxide Films

Metal	Oxidizing Conditions Temp °C.	Time Min.	Composition and *a* By transmission	By reflection	X-ray Data
Co	200	50	Co_3O_4, 8.07; CoO, 4.24	CoO, 4.28	....
	300	10	Co_3O_4, 8.06; CoO, 4.25	Co_3O_4, 8.12	Co_3O_4, 8.11
	400	5	CoO, 4.24	CoO, 4.28	....
	400	10	CoO, 4.23	CoO, 4.22	CoO, 4.25
	400	30	CoO, 4.24	CoO, 4.28	....
	500	5	CoO, 4.25	CoO, 4.29	....
Cu	200	5	Cu_2O, 4.23	Cu_2O, 4.32; CuO	Cu_2O, 4.24
	200	30	Cu_2O, 4.24	Cu_2O, 4.33	....
Fe	250	5	Fe_3O_4, 8.35	Fe_3O_4, 8.49	Fe_3O_4, 8.40
	250	30	Fe_3O_4, 8.35; α-Fe_2O_3	Fe_3O_4, 8.44; α-Fe_2O_3	....
	300	5	Fe_3O_4, 8.37	Fe_3O_4, 8.43	γ-Fe_2O_3, 8.32
Ni	400	20	NiO, 4.17	NiO, 4.20	....
	450	5	NiO, 4.15	NiO, 4.20	NiO, 4.17
	500	5	NiO, 4.15	NiO, 4.20	....
	500	20	NiO, 4.15	NiO, 4.22	....
	500	60	NiO, 4.17		
	550	5	NiO, 4.19		

If this effect is real, then the removal of the positive deviations in the stripped oxide film pattern is an interesting question. This may result from the electrochemical attack on the iron in solid solution in the oxide lattice or by removal of strains in the film after stripping from the metal. Support for the former hypothesis can be seen in the observations of Bernard (*2*) on the increase of the lattice parameter of FeO from 4.282 Å. to 4.298 Å. by the solid solution of iron in the lattice. This is of the same sign and magnitude as the positive deviations observed in Table VI.

The negative deviations are also of interest. They are greatest for Fe_3O_4 and Co_3O_4, where other oxides may be formed and go into solid solution, and are least for NiO and Cr_2O_3, where only one oxide is observed.

Electron Microscope. The size and shape characteristics of the crystals in an oxide film are determined by the

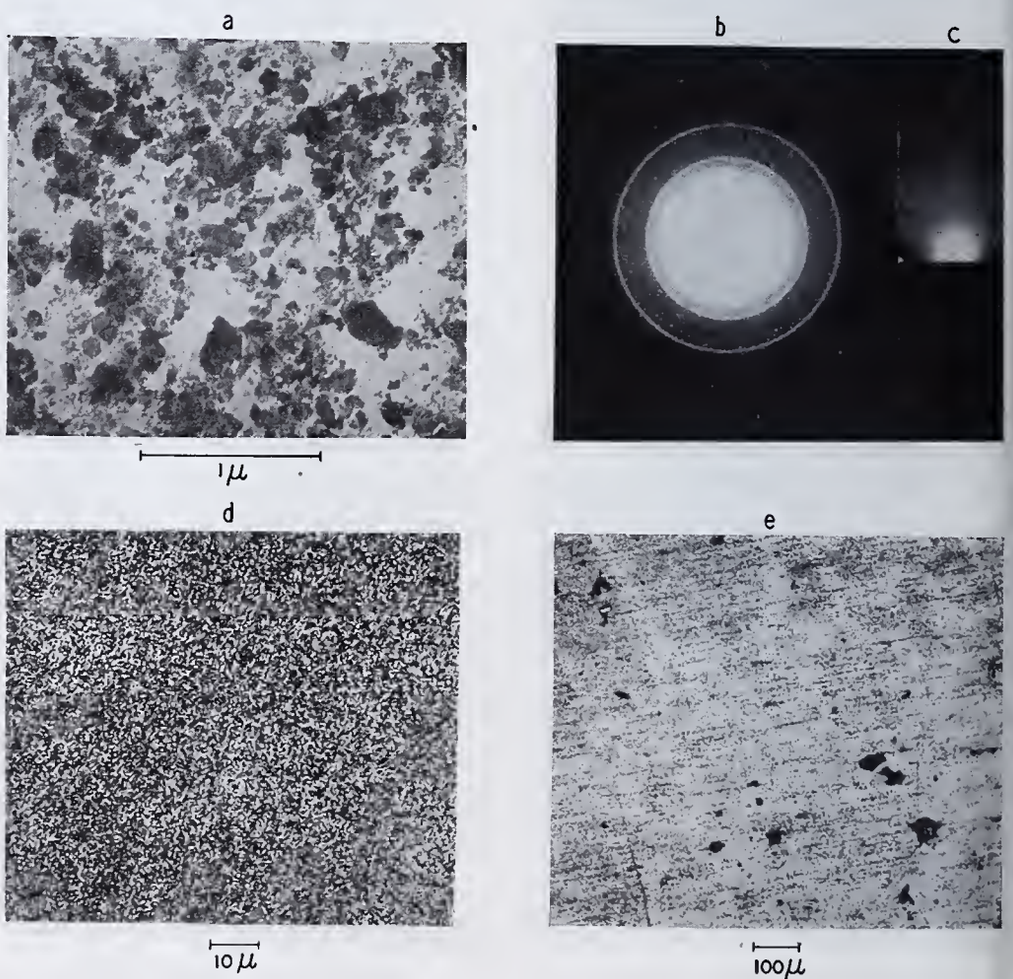


Figure 12. Oxide Film of Aluminum, Al 5–500

a. Electron micrograph stripped film
b. Electron diffraction transmission stripped film
c. Electron diffraction reflection film on metal
d. e. Light micrograph film on metal

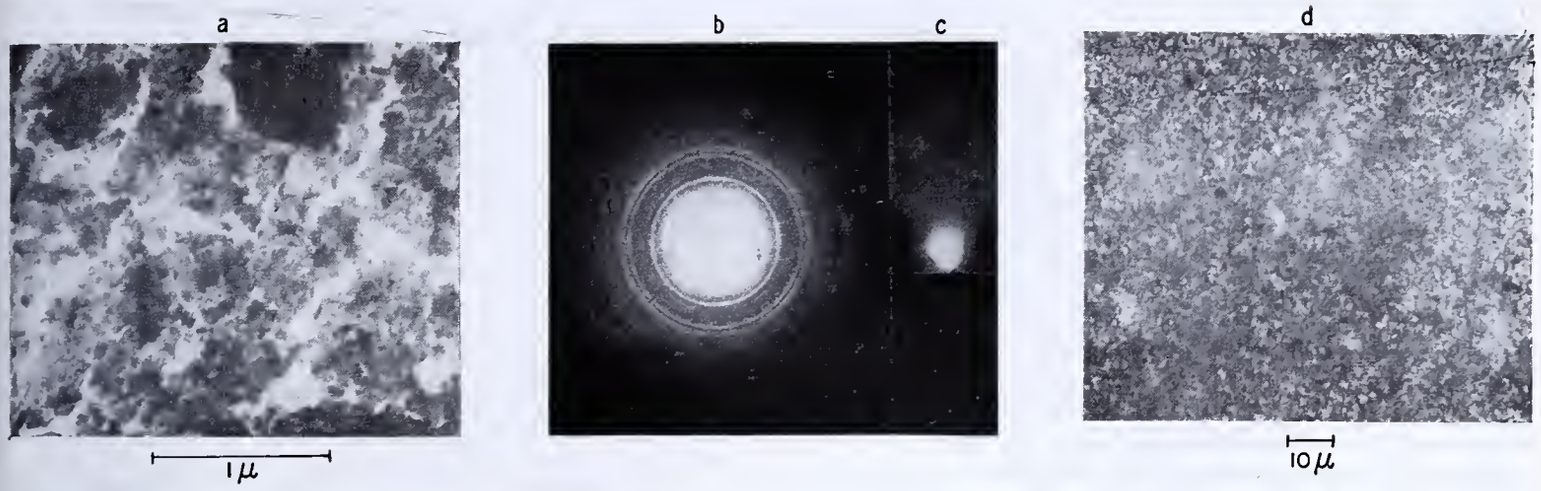


Figure 13 (*Above and Right*). Oxide Film of Columbium, Cb 5–400

a. Electron micrograph stripped film
b. Electron diffraction transmission stripped film
c. Electron diffraction reflection film on metal
d, *e*. Light micrograph film on metal

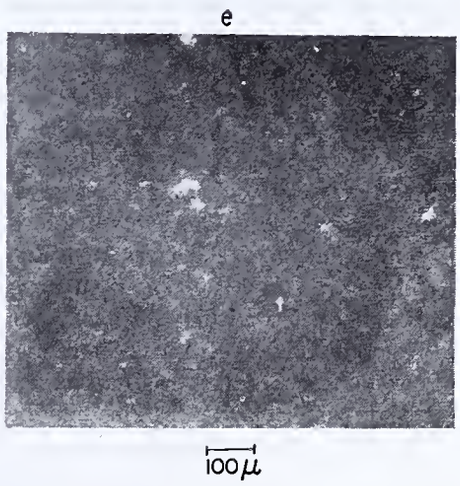


Table VI. Average Deviation of Oxide Lattice Parameters from X-Ray Values

	Transmission			Reflection		
Metal	No. of expts.	Composition	Deviation, %	No. of expts.	Composition	Deviation, %
Co	6	CoO	−0.20	5	CoO	+0.47
	2	Co_3O_4	−0.56	1	Co_3O_4	+0.12
Cu	2	Cu_2O	−0.12	2	Cu_2O	+2.0
Fe	3	Fe_3O_4	−0.55	3	Fe_3O_4	+0.63
Ni	6	NiO	−0.16	4	NiO	+0.84
Cr[a]	2	Cr_2O_3	+0.10	2	Cr_2O_3	+0.62
	Average deviation		−0.20%	Average deviation		+0.72%

[a] Calculated from d_{hkl} values.

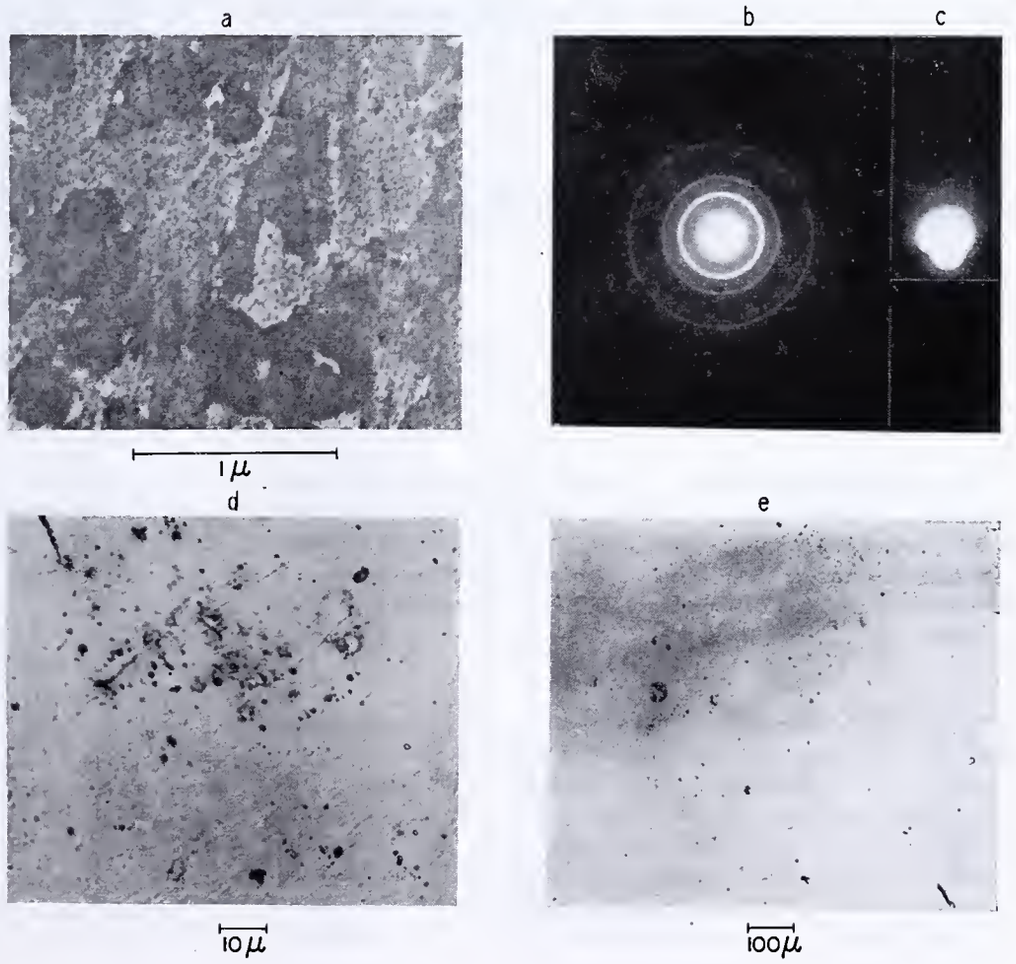
face. The number and size of the oxide crystals are therefore a function of the temperature. The shape, sharpness of the crystal boundaries, and other features of the oxide crystal are determined by the rate processes and the crystal structure of the oxide. Silica is known to be amorphous, while cuprous oxide is definitely crystalline.

Another point of view of the oxidation process which may be useful is a comparison of the volume ratio of the oxide to the metal. Metals which form oxides having a volume ratio greater than 1 are usually protective, while metals forming oxides with a volume ratio of less than 1 are usually nonprotective. If the crystals are to grow uniformly in all directions, compressive forces may be expected in one type of film and tensile forces in the other. This phenomenon has been used frequently to explain the porosity of some films and the cracking of others.

The process of formation of the first layer of crystals in the oxide film is interesting. The first stage of the reaction of a clean metal with oxygen has been shown to involve the formation of a uniform monomolecular layer of oxygen. A thin film of oxide has been found to grow rapidly after the initial layer has been formed (*9*). At some point crystal nucleation must start and

Figure 14 (*Below*). Oxide Film of Tungsten, W 5–400

a. Electron micrograph stripped film
b. Electron diffraction transmission stripped film
c. Electron diffraction reflection film on metal
d, *e* Light micrograph film on metal



then the crystal may grow. Its growth in the surface plane is limited by the interference of other crystals.

The electron micrographs and light micrographs of the oxidation experiments are shown in Figures 4 to 14. Table III summarizes the information taken from the electron micrographs. The films consist of small oxide crystals 100 to 2500 Å. in size, largely of irregular shapes with a few films showing definite crystal shapes. The oxide crystals are of the order of 10^{-3} to 10^{-5} of the linear dimension of the metal crystal or grain and 10^{-6} to 10^{-10} of the area.

The crystal size is a function of both time and temperature. The effect of temperature can be shown in the series of oxidation experiments on cobalt and on nickel. For the nickel oxidation series the average oxide crystal size increases from 400 Å. at 400° C. to 600 Å. at 550° C., while in the cobalt oxidation series the average oxide crystal size increases from 450 Å. at 200° C. to 1000 Å. at 500° C. The time effect on the oxide crystal size can be seen from an analysis of the cobalt, copper, and iron experiments.

The oxide films are largely nonuniform, as they consist of thicker and thinner sections. This indicates a multilayer film of oxide crystals. Thus, nucleation occurs in contact not only with the initial thin oxide layer but with other oxide crystals. This multilayer nucleation process does not form oxide crystals in as regular a manner as in the first layer. Therefore, clustering of crystals in the outer layers is noticed and nonuniform films are formed. Local concentrations of impurities or a small void in the initial oxide layer may partially account for this phenomenon.

It is noticed from an analysis of the electron micrographs that frequently one crystal appears to overlap an adjacent one, causing a broad boundary zone to appear. This may be the result of (1) the physical overlap of two crystals, or (2) occurrence of the contact zone between crystals at an angle to the electron beam. Diffraction effects may give the same effect as physical overlapping of the crystals. This overlapping phenomenon is noticed in many of the films.

The irregular shape of the oxide crystals in the first layer of the oxide film is to be expected. If a second or third layer of crystals is formed on top of the initial layer in a nonuniform manner, the previous restraints are relaxed and the new crystals may assume a more normal growth (Figure 6).

The oxides formed during the oxidation of columbium, tungsten, and chromium gave the smallest average crystal size. The largest crystals are formed during the oxidation of copper, iron, molybdenum, nickel, and cobalt. The first group probably form the more protective films against oxidation under the conditions of the experiment. If the materials are compared at the same temperature, nickel and cobalt would be included in the first group and not in the last. The more protective metals appear to form more uniform films, although the correlation is not general.

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Detection of Palladium Using Pararosaniline Hydrochloride

A Spot Test Procedure

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The reaction between palladous chloride and *p*-fuchsin has been studied. *p*-Fuchsin reacts with palladous chloride at a mole ratio of 2 to 3, apparently with the formation of a double salt. A spot test is proposed which is sensitive to 0.01 microgram of palladium at a limiting concentration of 1 part in 750,000. The reaction is highly selective.

DURING polarographic analyses in which pararosaniline hydrochloride was used as a maximum suppressor, it was observed that the pararosaniline hydrochloride color faded gradually upon the addition of palladous chloride. The investigation then under consideration included approximately one hundred different ions, and since the fading took place only in the presence of the palladous salt, it was thought that the reaction was sufficiently specific to warrant further study. In the investigation, emphasis was placed on the adaptation of the reaction for use as a spot test, although considerable effort was directed toward elucidating the nature of the reaction.

REAGENTS AND CHEMICALS

Schultz No. 511 pararosaniline hydrochloride (*p*-fuchsin) from the National Aniline and Chemical Company, Inc., was made up to a strength of 0.01%, and C.P. palladium chloride to 0.01% (in respect to palladium) in distilled water. One per cent solutions of substances to be studied for interfering effects were made from C.P. chemicals; 2 *N* sodium hydroxide and 2 *N* acetic acids were used in adjusting the hydrogen-ion concentration of the test solutions.

APPARATUS

Spectrophotometric studies were made using a Model D Beckman spectrophotometer and 1.00-cm. cells.

Table I. Comparison of *p*-Fuchsin, Dimethylglyoxime, and *p*-Nitrosodiphenylamine Tests for Palladium

	p-Fuchsin[a]	Dimethylglyoxime	*p*-Nitrosodiphenylamine
Positive interferences	Au^{+++}, Hg^{+}, Pt^{++++}	Au^{+++}	Au^{+++}, Pt^{++++}
Negative interferences	Tl^{+}, Bi^{+++}, CN^{-}, $C_2O_4^{--}$, basic NH_4^{+}, F^{-}, CNS^{-}, formate, aniline, pyridine	Be^{++}, Ga^{+++}, basic NH_4^{+}, BO_3^{-}, S^{--}, $S_2O_3^{--}$, CN^{-}, $Fe(CN)_6^{----}$, CNS^{-}, $C_2O_4^{--}$	Basic NH_4^{+}, NO_2^{-}, CN^{-}, $Fe(CN)_6^{---}$, $Fe(CN)_5^{----}$, CNS^{-},
Masking interferences	Sn^{++}, V^{+++}, MoO_4^{--}, I^{-}, Ir^{+++}, hypophosphite	Ti^{+++}, hypophosphite, V^{++++}, CrO_4^{--}, I^{-}, Fe^{++}, Fe^{+++}, Rh^{+++}, Ir^{++++}	Ag^{+}, Ti^{+++}, Sn^{++}, hypophosphite, VO_3^{-}, S^{--}, Cr^{+++}, CrO_4^{--}, MoO_4^{--}, I^{-}, Co^{++}, Ru^{+++}, Ir^{++++}
Sensitivity	LI = 0.01 microgram LC = 1:750,000	LI = 0.18 microgram LC = 1:100,000	LI = 0.005 microgram LC = 1:750,000

[a] Positive interferences listed refer to reactions with *p*-fuchsin. Actual test based on use of this reagent, however, is free from positive interferences.

EXPERIMENTAL

Figure 1 shows the transmittancy curves for solutions of pararosaniline hydrochloride, palladous chloride, and a mixture of these two solutions. These transmittancy curves were determined using 0.005% solutions of pararosaniline hydrochloride and 0.0005% palladous chloride. The reference cell contained distilled water. The red color of the fuchsin solution fades on addition of the palladium salt. The maximum transmittancy difference occurs at a wave length of 380 mμ. Visual observation of this reaction discloses that dilute solutions of palladium cause a fading of the red fuchsin color, while large concentrations of palladium react with the fuchsin to form a finely dispersed brown precipitate.

The nature of the reaction involved was next investigated. The equivalent ratio of the two reactants was determined by means of a spectrophotometric titration. This procedure was based on the observation that the slopes of the transmittancy *vs.* molar concentration curves for the fuchsin and the palladous chloride solutions were quite different when determined at a wave length of 380 mμ. By adding known amounts of standard 0.000999 *M* palladium solution to fixed amounts (5.00 ml.) of 0.000247 *M* *p*-fuchsin, diluting the mixture to a total volume of 10.00 ml., determining the transmittancies, and plotting the log per cent transmittancy of each mixture, it was found that the equivalence point, as indicated by the intersection of the two curves, was at a mole ratio of *p*-fuchsin to palladous chloride of 2 to 3 (see Figure 2). As a check on this determination, analyses for carbon, hydrogen, and chlorine were run which confirmed that the composition of the reaction product was $2C_{19}H_{17}N_3HCl.3PdCl_2$. Analytical calculations for $2C_{19}H_{17}N_3HCl.3PdCl_2$: C, 38.71; H, 3.07; Cl, 24.06. Found: C, 38.73; H, 3.43; Cl, 24.36.

The nature of the fading action due to palladium was different from the fading brought about by acids or reducing agents. This was indicated by the transmittancy curves of acid-faded and sulfur dioxide–faded *p*-fuchsin as shown by Figure 3 when compared to Figure 1.

On the basis of the analytical data presented, the authors believe that the product of the palladous chloride–pararosaniline hydrochloride reaction is a double salt. The probability that the fading action was due to oxidation-reduction was ruled out on the basis of the transmittancy curves. The possibility that an inner-complex salt was formed was rejected because the functional groups of the pararosaniline were so far apart as to prohibit the formation of stable chelate rings. Normal salt formation was considered unlikely because the reaction occurred in slightly acidic medium. The belief that the reaction results in double salt formation is of interest, since this type of reaction has not previously been considered of analytical importance (*1*, *8*, *11*).

Because of the close similarity between members of the platinum group metals, special emphasis was placed on determining the possibility that other elements of this group might react with fuchsin in a manner similar to palladium. Transmittancy curves for mixtures of *p*-fuchsin and ions of ruthenium, rhodium, osmium, iridium, and platinum were run and it was found that the transmittancies of mixtures of the reagent with these ions were additive.

APPLICATIONS AS A SPOT TEST

There are several tests for palladium (*3–7*, *9*, *10*, *12*, *15*), the most sensitive of which is the *p*-nitrosodiphenylamine reaction (*15*). This test is sensitive to 0.005 microgram of palladium and there are few interfering substances. The method can also be used for quantitative determinations of the metal.

The authors have investigated *p*-fuchsin as a reagent for detecting palladium and have found it to be highly selective in its reaction. Most of the important interferences can be eliminated by simple means and since *p*-fuchsin is a common stable organic compound, and the test is so sensitive and easy to make, this method of testing for palladium should find considerable application in research and routine analytical laboratories.

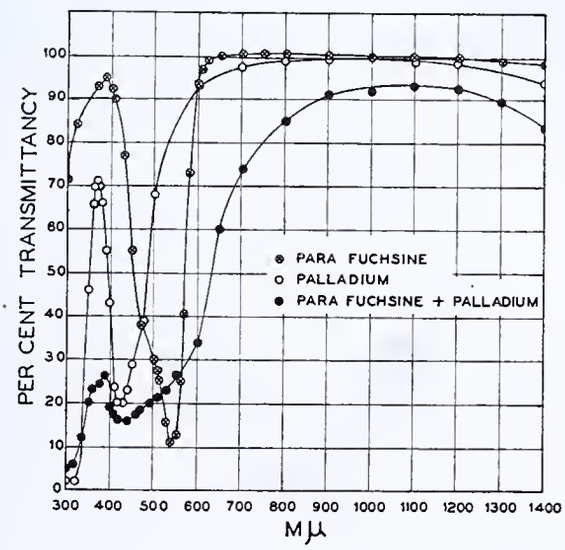

Figure 1. Transmittancy Curves

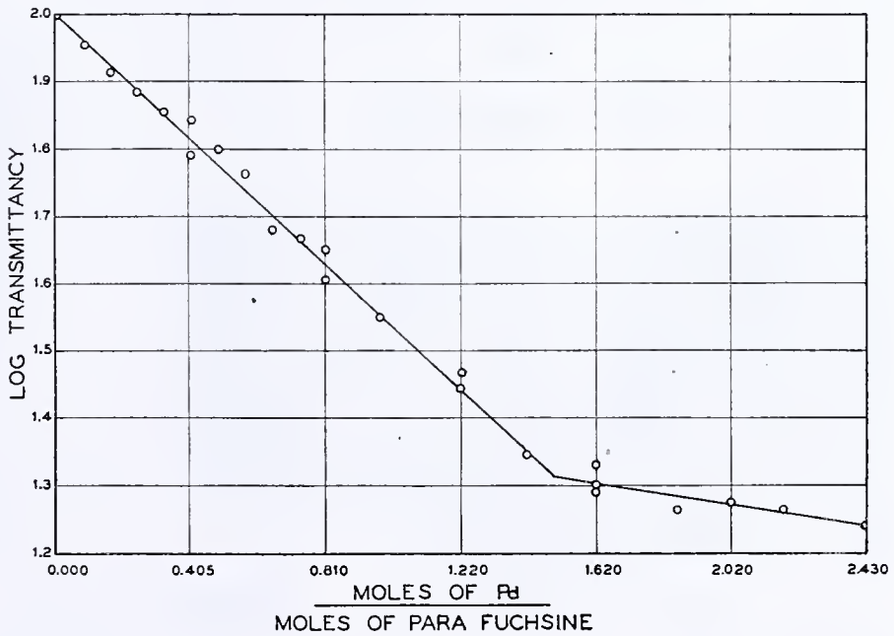

Figure 2. Equivalent Ratio of Reactants

SCOPE OF INVESTIGATION

The interference studies were made using 1% solutions of the ions and compounds listed below (the ions are listed in their most common forms, although it is realized that in many cases they are actually present as complexes):

Li^+, Na^+, K^+, Cu^{++}, Rb^+, Ag^+, Cs^+, Au^{+++}, Be^{++}, Mg^{++}, Ca^{++}, Zn^{++}, Sr^{++}, Cd^{++}, Ba^{++}, Hg^+, Hg^{++}, BO_2^-, $B_4O_7^{--}$, Al^{+++}, Ga^{+++}, Y^{+++}, In^{+++}, La^{+++}, Ce^{++++}, Tl^+, CO_3^{--}, SiO_3^{--}, Ti^{++++}, Sn^{++}, Sn^{++++}, Pb^{++}, Zr^{++++}, Th^{++++}, NH_4^+, NO_2^-, NO_3^-, $H_2PO_2^-$, $P_4O_{13}^{------}$ (tetraphosphate), $P_6O_{18}^{------}$ (hexametaphosphate), PO_3^-, HPO_4^{--}, $P_2O_7^{----}$, VO_3^-, AsO_2^-, $HAsO_4^{--}$, Sb^{+++}, Bi^{+++}, S^{--}, $S_2O_3^{--}$, SO_3^{--}, SO_4^{--}, Cr^{+++}, CrO_4^{--}, SeO_3^{--}, SeO_4^{--}, MoO_4^{--}, WO_4^{--}, TeO_3^{--}, UO_2^{++}, UO_4^{--}, F^-, Cl^-, ClO_3^-, Mn^{++}, MnO_4^-, Br^+, BrO_3^-, I^-, IO_3^-, ReO_4^-, Fe^{++}, Fe^{+++}, Co^{++}, Ni^{++}, Ru^{+++}, Rh^{+++}, OsO_5^{--}, Ir^{++++}, Pt^{++++}, CN^-, $Fe(CN)_6^{---}$, $Fe(CN)_6^{----}$, SCN^-, acetate, oxalate, malonate, formate, adipate, succinate, phthalate, tartrate, citrate, lactate, gluconate, isoinositol, *d*-sorbitol, mannitol, sucrose, dextrose, aniline, pyridine, resorcinol.

The pH of solutions made acidic by the addition of a drop of solution of such salts as Ti^{++++} and Sn^{++} was adjusted to a value of between 2 and 4, as indicated by universal indicator paper, by adding 2 to 3 drops of sodium hydroxide and then acetic acid until the desired pH was obtained. Usually 2 to 3 drops of acetic acid sufficed. The ratio of the substance being studied for interference to the palladium was 100 to 1 (*14*). This unfavorable ratio represented a severe study of the test and indicated what could be expected of it under ordinary working conditions.

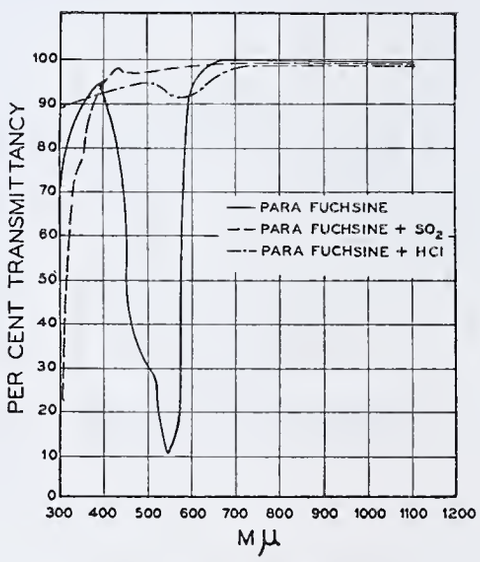

Figure 3. Transmittancy Curves

The test was carried out by placing one drop of palladium and one drop of distilled water in a depression of a spot plate. In an adjacent depression was placed one drop of a solution of the substance being studied for interference plus one drop of palladium solution, and to a third consecutive depression were added one drop of the substance and one drop of distilled water. This procedure ensured approximately equal concentrations of each reagent in any test solution. Into each of the three depressions one drop of the *p*-fuchsin was then measured. A faded brown color or a brown precipitate, depending on the concentration, constituted the test. Where the solution of the substance being studied was so acidic as to fade the *p*-fuchsin, the pH was adjusted as described above and then the volume of the solution containing only palladium salt was adjusted with distilled water before the *p*-fuchsin was added.

An investigation of the limit of identification and the limiting concentration (*2*) of the *p*-fuchsin test for palladium showed that the limit of identification was 0.01 microgram at a limiting concentration of 1 to 750,000. These limiting values were obtained using micro volumes of both the reagent and the palladium test solutions. The value for the limiting concentration does not include any volume increases due to the addition of reagents.

The interferences were classified in the manner described by West (*13*).

Positive interferences were given by auric gold, mercurous mercury, and large amounts of platinic platinum. These positive interferences can be obviated by making a confirmatory test with crystalline sodium hypophosphite. The hypophosphite reduces palladium in any solution with the formation, after a few seconds, of a black precipitate. Solutions containing gold or platinum alone are not visibly affected by hypophosphites, while solutions of mercurous mercury give a purple precipitate and this only upon standing. This confirmatory test should be run on solutions containing *p*-fuchsin, since otherwise mercurous mercury gives a black precipitate similar to that given by palladium. The reaction between platinum and *p*-fuchsin gives a visible effect only in solutions which contain over 5 mg. of platinum per ml.

Negative interferences were given by ammonium hydroxide, thallous thallium, nitrite, bismuth, cyanide, oxalate, and, upon standing (4 hours), fluoride, thiocyanate, formate, aniline, and pyridine. With the exception of bismuth, these negative interferences are due to competitive reactions which so reduce the effective concentration of palladium that it no longer gives its characteristic reaction with *p*-fuchsin. In the case of thallium, the reaction results in the formation of a precipitate, while in the case of the other ions, soluble complexes are formed. As a rule, these negative interferences would apply to any test for palladium.

Masking interferences were given by stannous tin, vanadous vanadium, hypophosphite, permanganate, iodide, and iridium. The tin, hypophosphite, and iodide react with palladium to give dark precipitates, while the other ions interfere, owing to the intense colors of their solutions. Of these interferences, the only one of practical significance is that of iridium; it can be eliminated by addition of thiosulfate until the color of the iridium just disappears. Excess thiosulfate should be avoided. Iridium in concentrations of less than 5 mg. per ml. does not mask the *p*-fuchsin test.

A comparison of the *p*-fuchsin test with the dimethylglyoxime and *p*-nitrosodiphenylamine tests for palladium is shown in Table I. All three tests were very satisfactory. Advantages claimed for the *p*-fuchsin test are that the positive interferences can be very easily obviated, and that no interferences of any type are given by other members of the platinum group of metals, provided that the initial concentration of the solution to be analyzed does not exceed 0.05%. Because of the nature of the test, it suffers least from masking interferences due to colored ions.

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Spectrographic Analysis of Zinc-Base Alloys

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A rapid and accurate spark spectrochemical system incorporating the use of the ammonium chloride–packed electrode and the flat disk self-electrode has been developed. A commercial high-voltage spark source unit with extra inductance is used for excitation of flat disk samples on a Petrey spark stand. A grating spectrograph is employed. Comparator-densitometers are used for transmission measurements and conversion to intensity ratios is made on calculating boards. The system provides for simultaneous evaluation of all zinc alloy constituents and impurities quantitatively on one spectrogram. The method accurately measures lead in the range of 0.002 to 0.015, tin 0.002 to 0.015, cadmium 0.002 to 0.010, magnesium 0.01 to 0.10, iron 0.01 to 0.10, copper 0.75 to 3.25, and aluminum 3.50 to 4.50%.

THE accurate and complete analysis of zinc-base alloys for all alloy constituents and impurities by chemical methods is tedious, time-consuming, and often subject to serious discrepancies. Using the accepted chemical methods of analyses for this type of alloy, rapid routine determinations are difficult, since the chemist often has to approach his analytical problems as if they were of umpire quality. For some analyses he has to use large samples, make multiple precipitations, exercise the utmost care, and hope that his result truly represents his sample.

Zinc-base alloys are manufactured under one of the closest specification allowances of any alloy blended today; the specification prohibits the presence of more than 0.005% cadmium and similarly low amounts of tin and lead in the alloy. The spectrograph therefore offers emancipation from the drudgery of difficult and sometimes questionable chemical analyses.

Two and a half years ago the laboratories of Morris P. Kirk & Son, Inc., subsidiary of the National Lead Company, acquired spectrographic equipment for the express purpose of controlling the production of nonferrous metals and alloys. These alloys consist of tin-base, lead-base, zinc-base, and a wide range of aluminum alloys. Today over 75% of all these analyses are made spectrographically, thanks largely to the development of a special, but simple, electrode.

This special electrode (Figure 1) introduces ammonium chloride into the spark discharge from its tip, where the salt has been placed in a hole drilled in the electrode tip. These ammonium chloride–packed electrodes are processed from National Carbon Company "spectroscopic carbons" by means of a special cutting tool, which simultaneously fashions a hemispherical tip and drills a hole 0.156 cm. ($^1/_{16}$ inch) in diameter and 0.234 cm. ($^3/_{32}$ inch) in depth. This cutting tool is chucked in a high-speed electrode machine, which contributes ease and speed to quantity production of electrodes. The electrode may be prepared without the services of the special tool by cutting a hemispherical tip with a standard tool, then slightly blunting the tip with a fine file and by means of a $^1/_{16}$-inch drill, mounted in a sleeve, drilling the hole to the desired depth. It is essential that care be observed in electrode fabrication concerning the position of the periphery of the tip. The electrode tip, as outlined by the periphery of the drilled hole, must be at right angles to the side of the electrode; if this condition is not attained the spark becomes deflected and produces an irregular pattern on the sample disk. This deflection invariably causes discordant results. However, a cutting tool with the drill mounted in the center of the cutting blades produces a perfectly formed electrode, requiring no further corrections.

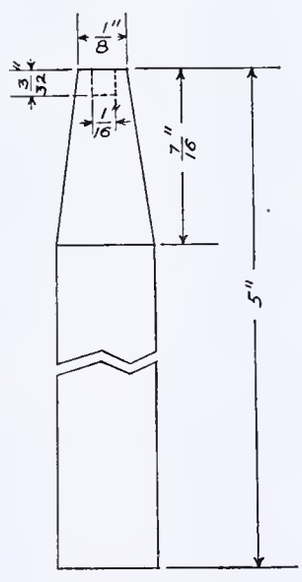

Figure 1. Hollow-Tipped Special Electrode

The spectrochemical system of analysis employed for the production control of the zinc-base alloys has been chosen for presentation here, rather than other problems handled by this method, because of the unusual requirements in analysis of high and low levels of alloy content and the very low level of impurities allowed. All constituents, alloying elements, and impurity elements are evaluated as a simultaneous quantitative estimation on one spectrogram.

The alloy under discussion, often referred to as No. 2 Zamak, contains aluminum 3.50 to 4.60%, copper 2.50 to 3.50%, magnesium 0.02 to 0.10%, iron 0.10% maximum, cadmium 0.005% maximum, tin 0.005% maximum, and lead 0.007% maximum. The literature offers few spectrochemical methods for the analysis of this type of alloy. Indeed, for the zinc-base alloys no method, including that of the A.S.T.M., provides for the quantitative determination of alloy constituents and the very low concentrations of allowable impurity elements in a single spectrographic operation, involving the spark technique. This is accomplished with the flat disk technique utilizing the Petrey spark stand, ammonium chloride–packed counterelectrode, and high-voltage spark source with extra inductance.

The method employed in the spectrochemical analysis of zinc-base alloys involves sample procurement and preparation, electrode preparation, excitation of sample and registration of spectral lines, film preparation, densitometry, and calculations.

SAMPLES AND PREPARATION

Samples for analysis may be specimens of any convenient size that have sufficient dimensions to rest on the Petrey stand. However, chill-cast disks measuring 2.5 inches in diameter and 0.5 inch in thickness have been found convenient to process in the laboratory. Figure 2 is a photograph of chill-cast disk and packed electrode. Die castings, parts of gates, sprues, and sawed slices from metal ingots have been utilized as samples for this type of alloy. Nondestructive analyses are often made on smooth casting surfaces with surprising accuracy. However, standard practice in this laboratory is to impart a smooth surface to each sample by means of the lathe cutting tool.

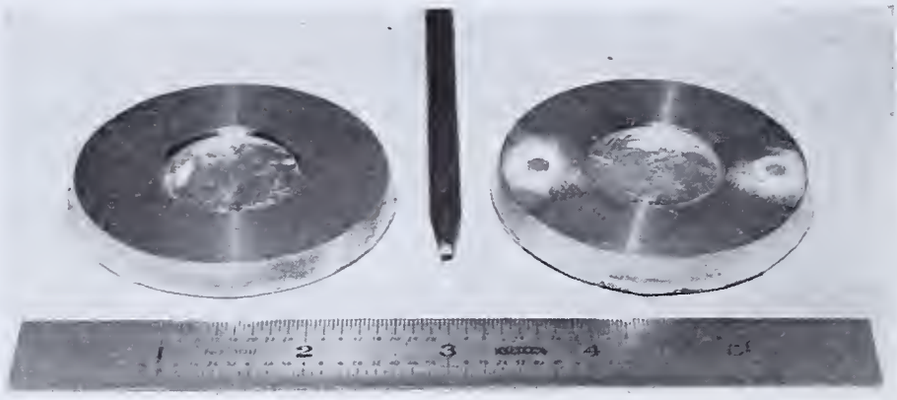

Figure 2. Chill-Cast Disk and Packed Electrode

$$\text{Intensity ratio } \frac{\text{Pb 2833 Å. + background}}{\text{Zn 2760 Å. + background}} -$$

$$\text{intensity ratio } \frac{\text{background at 2833 Å.}}{\text{Zn 2670 Å. + background}} =$$

$$\text{intensity ratio } \frac{\text{Pb 2833 Å.}}{\text{Zn 2670 Å + background}}$$

SPARK EXCITATION CONDITIONS AND FILM DEVELOPMENT

The special, hollow-tipped electrode is packed with the ammonium chloride salt by tamping it into a dish of the ground salt and smoothing flush with the periphery tip by means of a spatula or fine file. The fully prepared electrode is then placed in the lower clip-holder of the Petrey stand and adjusted for a 3-mm. gap by the swing-away gage. The smoothly machined disk, ingot slice, or casting segment is placed on the stand as the upper electrode.

The A.R.L.–Dietert, high-voltage spark unit is set at full power (2 kva.), and use is made of an additional inductance so that 1.46 millihenrys are available for the system. Excitation is made on an A.R.L.–Dietert grating spectrograph possessing a dispersion of 7 Å. per mm. Two 30-second sparking operations are performed on different parts of the sample surface. A new electrode is used for each excitation and the two 30-second exposures, without prespark, are superimposed on one spectrogram.

Registration is made on Eastman Spectrum No. 2 film which is processed 3 minutes in D19 developer at 16° C. The film is taken through all stages of processing under constant-temperature conditions and in rocker-type developer trays. A new electrode is employed for each 30-second excitation to give identical conditions of superimposition, because it was found desirable to maintain maximum gassing conditions over the full 60 seconds of sparking. Prespark is eliminated, because of the initial volatility of such elements as cadmium and lead.

The film calibration curve is obtained by sparking a standard iron sample for 1 minute, using the step-sector method. A single screen of 200-mesh wire cloth is placed before the lens to lessen the intensity and the high inductance unit is maintained in the circuit with output power dropped to 4/3 kva. Transmission values are selected from the 2800 to 3000 Å. region, and a plot of these data on log-log paper, using transmissions as ordinates and intensities as abscissas, derives the film calibration curve.

DENSITOMETRY AND CALCULATIONS

An exposure of one minute for zinc alloys produces a background which has to be accounted for in the calculations and the accurate measurement of the difference between line with underlying background, and background is utilized to measure the extremely small percentages of tin, cadmium, and lead encountered. Densitometry, therefore, includes measurement of transmission values for line plus background and adjacent background. The internal standard method of quantitative analysis is employed and all transmissions are referred to the 2670 Å. zinc internal standard line. All films are measured on two A.R.L.–Dietert comparator densitometers which are used interchangeably.

Calculations are made on calculating boards, and each element's spectral line, with underlying background and average adjacent background, is compared with the internal zinc line plus background.

A simple subtraction of background intensity ratio, from line plus background intensity ratio, gives the line intensity ratio corrected for background. No effort is made to measure the intensity of the 2670 Å. zinc line without background, since this line's background intensity becomes a constant in the calculation, and can be included with line intensity for all ratio derivations. A schematic presentation for the 2833 Å. lead line is:

Background readings, after some experience and practice, are made rapidly and the calculation of dual intensity ratios with a simple subtraction does not materially burden this phase of the analysis.

For the analysis of the copper-bearing, zinc-base alloys the following spectrum element lines have been found satisfactory:

	Å.
Zinc (internal standard)	2670
Cadmium	2288
Lead	2833
Magnesium	2928.7
Iron	2967
Aluminum	3060
Tin	3175
Copper	3194

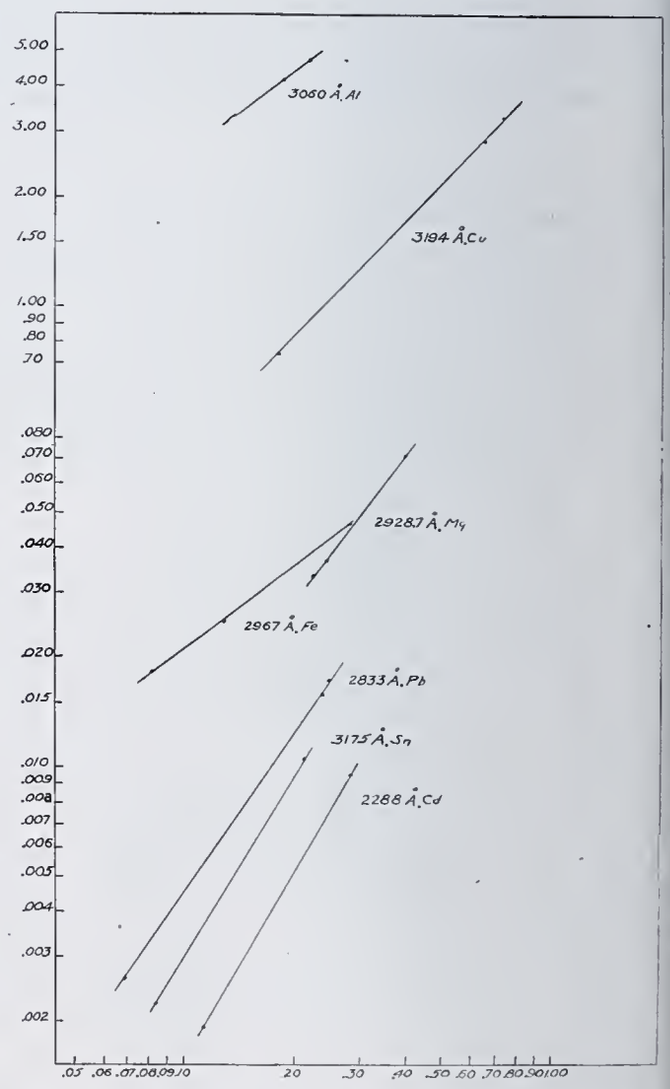

Figure 3. Working Curves Derived from Spectrochemical Data of Table II

Curves are valid for quantitative determination of aluminum and copper in Nos. 2 and 5 Zamak and for quantitative determination of magnesium, iron, lead, tin, and cadmium in Nos. 2, 5, and 3 Zamak

Table I. Typical Transmission Data, Lot 2142

	Zinc, 2670 Å.	Cadmium, 2288 Å.	Lead, 2833 Å.	Magnesium, 2928.7 Å.	Iron, 2967 Å.	Aluminum, 3060 Å.	Tin, 3175 Å.	Copper, 2194 Å.
Transmission of line plus background	11.4	43.5	35.7	22.8	45.5	43.5	64.3	39.9
Transmission of background	..	56.0	43.0	71.0	64.0	68.0	70.5	71.0
Intensity ratio-line plus background	..	0.280	0.350	0.545	0.287	0.278	0.159	0.31
Intensity ratio of background	..	0.200	0.280	0.130	0.160	0.142	0.133	0.13
Intensity ratio of line	..	0.080	0.070	0.415	0.127	0.136	0.026	0.180

Table II. Data for Plotting Working Curve

Lot No.		Cadmium	Lead	Magnesium	Iron	Aluminum	Tin	Copper
2142	Duplicate intensity ratios	0.080	0.070	0.415	0.127	0.136	0.026	0.180
		0.078	0.068	0.393	0.129	0.136	0.026	0.180
		Av. 0.079	0.069	0.404	0.128	0.136	0.026	0.180
	Chemical analysis, %	0.00097	0.0026	0.071	0.025	3.30	Trace	0.74
2200	Duplicate intensity ratios	0.290	0.237	0.220	0.291	0.181	0.218	0.750
		0.280	0.237	0.233	0.276	0.188	0.210	0.730
		Av. 0.285	0.237	0.226	0.283	0.185	0.214	0.74
	Chemical analysis, %	0.0094	0.0157	0.033	0.046	4.13	0.0103	3.25
2201	Duplicate intensity ratios	0.118	0.250	0.248	0.079	0.215	0.085	0.640
		0.109	0.245	0.243	0.085	0.222	0.080	0.670
		Av. 0.114	0.248	0.246	0.082	0.219	0.083	0.66
	Chemical analysis, %	0.0019	0.0172	0.037	0.018	4.70	0.0022	2.83

Table III. Comparison of Spectrographic and Chemical Analytical Results, Lot 2198

	Cadmium	Lead	Magnesium	Iron	Aluminum	Tin	Copper
Duplicate intensity ratio	0.145	0.138	0.235	0.285	0.180	0.105	0.724
	0.150	0.140	0.233	0.300	0.181	0.100	0.735
	Av. 0.148	0.139	0.234	0.292	0.181	0.103	0.730
Spectrographic, %	0.00295	0.00722	0.034	0.047	4.08	0.00312	3.18
Chemical, %	0.00280	0.00730	0.032	0.045	4.13	0.00297	3.25

COMPARATIVE ANALYSIS OF A ZINC-BASE ALLOY BY SPECTROCHEMICAL AND CHEMICAL METHODS

Three standard samples covering the compositional range of Zamak No. 2 and with sufficient impurity variations were selected to establish working curves for the spectrochemical analysis of a fourth sample designated "unknown". The analyses of each of the three standard samples, lots 2142, 2200, and 2201, are listed in Table II, with the duplicate intensity ratios derived for the respective element spectral lines by spark excitation of the three standards and one unknown, all in duplicate. The eight spectrograms comprising this film were made as in daily routine procedure and no undue precautions were exercised in the execution of the steps of the analysis.

The three standard samples and the unknown sample were very carefully analyzed in duplicate by two or more chemists, each of whom practiced precise and approved methods of chemical analysis, such as those sponsored by the New Jersey Zinc Company research laboratories. Large samples were employed for the analysis of low percentage impurity elements lead, tin, and eadmium, and for these elements more than one method of analysis was used.

Table I presents typical spectrochemical data derived from the spark excitation of No. 2142, one of the standard samples. Table II lists the individual average element intensity ratios and the corresponding chemical compositions for each standard sample used in this analysis. The working curves shown in Figure 3 were derived from plotting the values in Table II. An interesting and gratifying feature of the working curves is the accuracy with which all points fall on straight lines. Table III presents the results of the analysis on the unknown lot 2198 by spectrochemical and chemical methods.

A consideration of the average arithmetical per cent error in determinations of the various elements reveals that the major constituents, copper and aluminum, were measured well within an error of 3% of the amount present. The error expressed as per cent deviation from the chemical value of each element is: magnesium 6.2%, cadmium 5.3%, tin 5.0%, iron 4.4%, copper 2.1%, aluminum 1.2%, and lead 1.0%. The 6.2% deviation for magnesium might at first glance appear high enough to invalidate the worth of this determination. However, when the figure 6.2% is arithmetically applied to the accepted value of 0.032% we obtain a difference of 0.00198%, which, if equaled in routine chemical analyses, would be eonsidered satisfactory. The accuracy of the cadmium evaluation could, of course, be helped materially by the use of a ealibration curve constructed from transmission results taken in that region of the spectrum. The yardstick phrase "measuring an element within an error of 3 to 5% of the amount present" loses some of its comparative signification when applied to an element such as tin, which, in the case under consideration, is present in the amount of only 0.00297%. We can, therefore, accept higher values than 5% when we seek to evaluate elements present in a eoncentration on the order of 0.003%, since in the case of the tin evaluation in this analysis 5% of 0.00297% is 0.000148%, a negligible quantity in routine control analysis. A decrease in the accuracy of analysis at low concentrations is to be expected, particularly at levels where the difference between line with underlying background intensity and background intensity is small compared to baekground.

ANALYSIS TIME REQUIREMENTS

Sample procurement and sample preparation are in many spectrochemical methods great consumers of time. The flat disk technique simplifies sample procurement and, since no chemical treatment is necessary in sample preparation (only simple lathe machining), very rapid treatment is possible.

An analysis time of 5 to 7 minutes per element is easily achieved for a single sample; under normal routine conditions an analysis time of 2 minutes per element is achieved for a full film of seven unknown samples and one standard sample. Seven elements, of course, are evaluated for both the full film containing seven spectrograms and the film with only one spectrogram.

If one were to double these routine spectrochemical production time intervals of analysis per element, a comparison with time requirements for the chemical analysis of certain elements would still be meaningless; one cannot compare 30 minutes to 12 hours, which are the time requirements for determining cadmium. The 30-minute figure can be used for the spectrochemical evaluation of cadmium only if all film processing time is charged against one determination; 12 hours is a charitable time interval for a precise chemical cadmium determination by approved methods for the No. 2 Zamak alloy.

Iron, copper, and lead can be rapidly analyzed by chemical and electrolytic methods. Aluminum, magnesium, cadmium, and tin are the difficult time-consuming chemical determinations.

When the analytical problem is placed on a volume basis where many samples are handled each day for complete analyses, the spectrochemical method, as set forth in this paper, gains in attractiveness from the time-requirement standpoint.

ACCUMULATED DATA FROM LABORATORY CONTROL WORK

It is standard practice in this laboratory to assemble day to day analytical data on prospective standards before placing them

in service in the capacity of control standards. Opportunity is therefore offered to present data on the lead content of lot 2198, a standard sample which has been used on numerous films, with standard lot 2200, used in the analysis described above. Some 21 separate spectrochemical values for lead are presented which are, in all instances, based on the working curve shift from day to day as dictated by the 2200 standard. Lot 2199 was accurately determined by chemical methods to contain 0.0107% lead. The assembled data are given in Table IV.

In a similar manner data are available to show the day by day results for aluminum, the constituent present in the alloy in highest percentage (Table V). Lot 2144 was placed on many films along with standard lot 2200, not for the purpose of reliability checks, but rather for the more accurate evaluation of aluminum in the lower copper-bearing zinc alloys. At certain times it is expedient to place both high and low copper-bearing zinc alloys on the same film. The accurate and accepted chemical value for No. 2144 is 4.00% aluminum.

It is significant that lot 2200, which was used as the standard, contains 3.25% copper and that lot 2144 contains 1.00% copper. Experimental and production work has shown that in the analysis of zinc alloys for aluminum, standard sample and unknown sample should contain copper in similar amount. Consequently, the author never attempts to evaluate aluminum in No. 3 Zamak (copper-free) on the basis of a copper-bearing standard.

Table IV. Determination of Lead

Date of Analysis	Spectrochemical	Difference from Chemical Value of 0.0107%
	%	%
January, 1945	0.0115	0.0008
	0.0115	0.0008
	0.0110	0.0003
	0.0112	0.0005
March, 1945	0.0117	0.0010
	0.0110	0.0007
	0.0112	0.0005
	0.0108	0.0001
	0.0107	....
April, 1945	0.0096	0.0011
	0.0107	....
May, 1945	0.0102	0.0005
	0.0100	0.0007
	0.0105	0.0002
	0.0105	0.0002
June, 1945	0.0101	0.0006
	0.0105	0.0002
	0.0098	0.0009
	0.0114	0.0007
	0.0112	0.0005
	0.0098	0.0009
Average lead content, %		0.01066
Average per cent difference, %		0.00053
Deviation (% of quantity measured)		5.0
Standard deviation, %		0.000639

Table V. Determination of Aluminum

Date of Analysis	Spectrochemical	Difference from Chemical Value of 4.00%
	%	%
August, 1944	4.13	0.13
	4.05	0.05
	4.22	0.22
November, 1944	4.03	0.03
	3.88	0.12
	4.11	0.11
January, 1945	4.13	0.13
	4.10	0.10
	4.12	0.12
February, 1945	4.22	0.22
	3.92	0.08
	4.11	0.11
	4.13	0.13
March, 1945	4.13	0.13
	4.03	0.03
Average aluminum content, %		4.09
Average per cent difference, %		0.114
Deviation (% of amount measured)		2.85
Standard deviation, %		0.13

DEVELOPMENT OF AMMONIUM CHLORIDE–PACKED ELECTRODE

The development of the ammonium chloride electrode resulted from research on the use and effect of chemical salts in the spectrochemical analytical system involving the spark technique. Incentive for this type of research emanated from a desire to gain increased sensitivity and yet retain the speed and accuracy of the spark method.

Initial efforts to analyze flat disk zinc alloy samples using plain hemispherical tipped electrodes indicated that long exposure would be necessary to bring the transmission of the 2833 Å. lead spectral line to a measurable level. When total exposure was increased to include three and four 30-second intervals of spark excitation superimposed on one spectrogram, difficulties arose which, under the prevailing conditions, were insurmountable. Undesirable effects related to the high volatility of zinc resulted in erratic reproducibility of spectral-line intensity ratios for all elements. Backgrounds became very dense and in the cases of the low percentage elements, insufficient difference resulted between line and background intensities to give quantitative accuracy.

Ammonium chloride–packed electrodes, used in the first experimental runs as they are used in routine work today, lessened background, increased contrast between line and background, and produced a high degree of reproducibility.

The use of chemical salts in spectrochemical analysis is not new. However, in so far as the author has been able to discover, its use in the spark method is a radical departure. Hasler and Harvey (*1*) describe a method incorporating ammonium chloride in an arc analysis of zinc alloy. A metal peg of the zinc alloy is encased in ammonium chloride and contained in a graphite sleeve holder. This assembly becomes the lower electrode and graphite is utilized as the upper electrode. Four seconds' arcing time is employed and, according to the authors, a large amount of sample is energized in the discharge. A good degree of accuracy and reproducibility is indicated by the published results.

SUMMARY

A rapid and accurate spark spectrochemical system incorporating the use of the ammonium chloride–packed electrode and the flat disk self-electrode has been developed. A commercial high-voltage spark source unit with extra inductance is used for excitation of flat disk samples on a Petrey spark stand. A grating spectrograph is employed and spectral line registration is made on Eastman No. 2 film. Comparator-densitometers are used for all transmission measurements and conversion to intensity ratios is made on calculating boards.

The system provides for the simultaneous evaluation of all zinc alloy constituents and impurities in a quantitative manner on one spectrogram. The method is applicable to the Zamak series of zinc alloys over the full compositional range as well as to other types of alloys.

The method presented accurately measures the quantity of lead in the 0.002 to 0.015 range, tin in the 0.002 to 0.015 range, cadmium in the 0.002 to 0.010 range, magnesium in the 0.01 to 0.10 range, iron in the 0.01 to 0.10 range, copper in the 0.75 to 3.25 range, and aluminum in the 3.50 to 4.50% range.

Data presented in all instances represent results obtained in the routine execution of the method. Certain selective refinements of method manipulation could have been practiced in the

production of the data cited, but the true validity of the system is shown by results obtained under daily routine conditions.

The application of the ammonium chloride–packed electrode in the analysis of other types of alloys has necessarily had to suffer in this paper. However, better than 70% of all the author's spectrographic results are obtained by the use of this electrode, which has controlled the production of many thousands of tons of specification alloys for over two years.

The speed of sample preparation (nondestructive, if necessary), simplicity, and directness of manipulation combined with a high degree of analytical accuracy are advantages of the method.

ACKNOWLEDGMENT

The author wishes to express his thanks and appreciation to M. F. Hasler for his most helpful cooperation on the many problems which arose in the standardization of the method, and to B. M. Luckenbill of the Spectrographic Laboratory and E. Knapp of the Chemical Laboratory for their indispensable analytical work.

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Preparation of Synthetic Samples of Low-Boiling Hydrocarbons

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Field Research Department, Magnolia Petroleum Company, Dallas, Texas

The design and operation of an apparatus for the blending of low-boiling hydrocarbons are described. The apparatus is so designed that standard samples containing any number of components may be prepared rapidly, in any size, and with a degree of accuracy far superior to present methods of analysis. The apparatus has been thoroughly tested. Data are presented for samples in the butane and pentane range.

With the constantly increasing emphasis placed upon newer physical methods of hydrocarbon analysis (*2*)—i.e., infrared spectroscopy, mass spectroscopy, etc.—a growing need has been felt for synthetic mixtures of low-boiling hydrocarbons. Not only are such mixtures necessary as a final test upon calibration data but periodically a check sample must be run to ensure continued satisfactory performance of the instrument.

Several years ago the need for such a method became apparent in these laboratories. A literature search revealed very little in the way of blending units versatile enough to satisfy the varied requirements of the work. Not only were small "spot" samples required for infrared and mass spectrographic work, but large samples occasionally were necessary to develop adequately a variety of methods, ranging from Matuszak procedures and bromine numbers to low-temperature distillation analyses.

The usual practice for obtaining such samples has taken either of two forms. The sample may be prepared upon a pressure basis, putting each component successively into a constant-volume device and measuring the pressure increments added. Large volumes are necessary if the sample is to be of appreciable size and the introduction of inert material, either from a small leak in the system or from the hydrocarbons themselves, may introduce serious error. Deviations from the gas law equation may also be appreciable if mixtures containing components having a different number of carbon atoms are blended or if relatively high pressures are used.

In the second method the composition of a mixture is obtained by another method of analysis. The instrument or method tested may then be evaluated on the basis of a study of this so-called "secondary standard". The objections to such an evaluation are obvious, in that the evaluation of the tested method is limited to the accuracy of the method used in obtaining the sample composition. With the ready availability of the greater number of pure low-boiling hydrocarbons on the market today, direct preparation of the sample from pure materials is the only satisfactory method.

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The blending apparatus described by McMillan (*1*) for the testing of low-temperature distillation procedures approaches most closely the apparatus developed in this laboratory. The sample was prepared upon a pressure basis, inert material being removed prior to preparation in the manner described below. Although satisfactory for the purpose at hand, the blending unit lacked the extreme versatility required in the present-day analytical laboratory.

DESCRIPTION OF APPARATUS

A schematic drawing of the apparatus is shown in Figures 1 and 2.

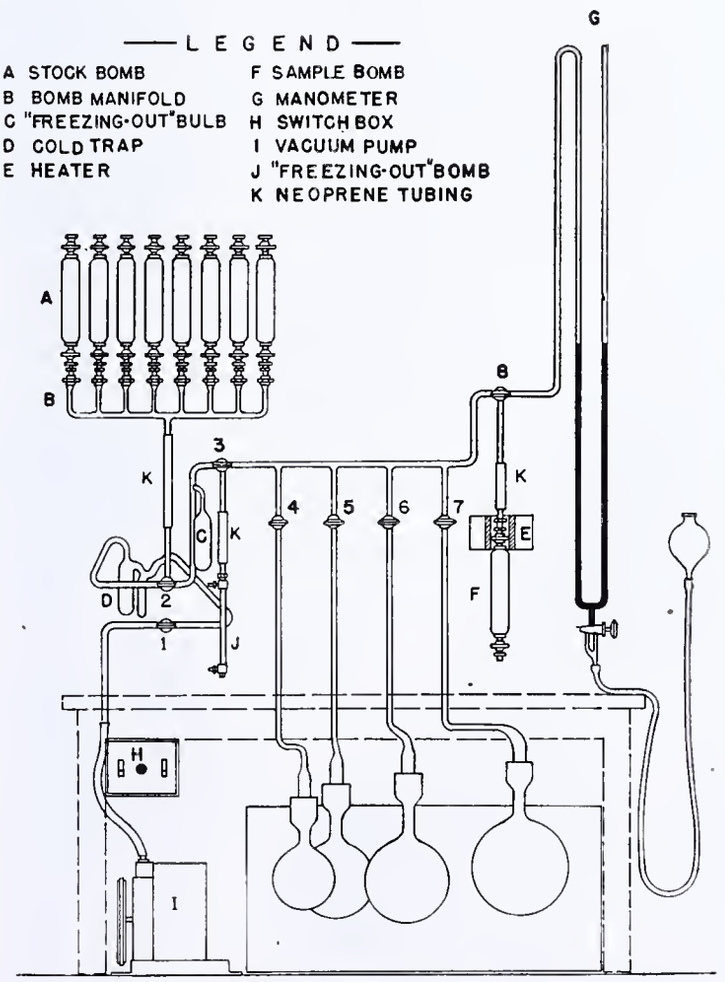

Figure 1. Schematic Drawing of Apparatus

Essentially the apparatus consists of the following:

A stainless steel bomb manifold, *B*, equipped with eight brass Hoke valves. To each valve is attached a brass compression fitting (for 1/4-inch pipe thread) similar to that shown in Figure 3.

A constant-volume manometer, *G*, complete with leveling bulb.

A 500-watt Calrod heater with shield, *E*, so arranged as to prevent freezing of hydrocarbon in the narrow neck of the enclosed Hoke valve.

A water bath surrounding four boiling flasks of the indicated sizes. Each flask is equipped with a mercury seal standard-taper joint to preclude leakage during sample make-up.

Three bomb types (Types 59, 65, 80), described in detail below.

A glass manifold equipped with the indicated stopcocks, "freezing-out" bulb, *C*, and liquid nitrogen traps, *D*.

A vacuum pump, *I*.

The apparatus is mounted as an integral unit on an angle-iron table having a 2.5-cm. (1-inch) Transite top. The manifold system and the expansion flasks are rigidly held in place by clamps leading from the various supports which, in turn, are securely bolted to the table top. A grill consisting of iron rod (0.5 inch) welded in 4-inch squares has been found most satisfactory for supporting equipment above the table top.

The apparatus measures 42 inches across the front and is 18 inches deep and 65 inches high.

SAMPLE BOMBS

A highly satisfactory type of small bomb has been evolved in this laboratory. Double-exit bombs have been found to be much superior to single-exit types on two important counts: They are more readily cleaned by flushing with the proper solvents and dried by passage of air current, and pressuring of samples is more readily accomplished.

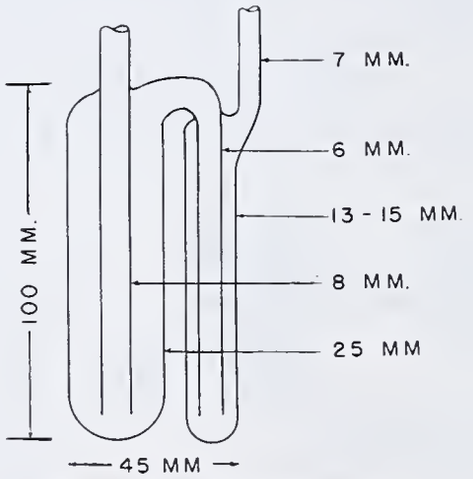

Figure 2. Liquid Nitrogen Trap

The Type 80 bomb, specially constructed for synthesis of hydrocarbon mixtures, consists of two brass Hoke valves threaded into a copper and brass body and then silver-soldered to form a gas-tight junction. Hoke valves with 1/4-inch pipe thread ends are used. The body is made of a 5.5-inch length of copper tubing (1.125 inches in outside diameter, 0.065-inch wall), inside which six vanes or fins are set longitudinally at 60° angles to each other. To the ends of this copper tube body are silver-soldered two brass swedge-type reducers. The assembly of this type of bomb is shown in Figure 3.

The inclusion of the vanes in the Type 80 bomb was found advantageous in achieving sufficient heat transfer during freezing out of hydrocarbon components. When an appreciable amount of hydrocarbon has been frozen into a bomb without such vanes, the interior of the mass tends to remain in a liquid-solid state for a considerable time and the vapor pressure of this mixture is appreciable. When the fin-type bomb is used, heat conduction is rapid enough to accomplish complete freezing throughout the mass, resulting in an appreciable reduction of the vapor pressure.

The Type 65 bomb is identical in dimensions and construction to the Type 80, except that the vanes are omitted.

The Type 59 bomb is composed of brass Hoke valves which have been machined at one end to fit a 4.5-inch length of copper tubing (0.5-inch outside diameter, 1/32-inch wall) and then silver-soldered in position (see Figure 3). Considerable material can be machined off the body of the valves to reduce materially the net weight of the bomb.

Each bomb is provided with two brass caps equipped with 16 gage sheet-lead liners which insure against loss of sample during storage.

A special brass compression fitting is used for connection to the 1/4-inch pipe thread of the valve-ends. Details of the cap and fitting are shown in Figure 3.

Chrome plating of these bombs is advantageous in that wear on the threaded surfaces and hence change in weight of the bomb are less. There is also less tendency to pick up stray mercury on the surface of the bomb.

CALIBRATION

Prior to preparation of synthetic samples, certain instrument constants must be obtained—the relative volumes of all flask and manifold combinations which may conceivably be used in sample make-up.

It has been found convenient to assign a value of unity to the volume occupied by the 500-ml. flask and the apparatus manifold. The manifold volume, in this case, includes all space from the reference mark of the manometer (explained below) to the Hoke valves of the steel bomb manifold, excluding the volume occupied by the sample bomb, the freezing-out bomb, and the liquid nitrogen traps. The relative volumes of all other flask combinations are thus stated in multiples of this so-called "unit volume".

All ratios are determined in a similar manner. Illustrative of the procedure is the determination of the ratio of the volume of the 1000-ml. flask to the unit volume.

Procedure. After testing the apparatus thoroughly for leaks, adjust the mercury columns in the manometer by means of the leveling bulb until the total of the readings in both arms is not more than 950 mm. and not less than 925 mm. (This is most important. Failure to observe this precaution whenever the system is evacuated may result in forcing mercury into the glass manifold.

With stopcocks 1, 4, and 5 open, stopcocks 3 and 8 by-passing the respective bombs, and stopcock 2 connecting all arms, evacuate the system. After the system is evacuated, close stopcock 1 and adjust the mercury column in the left arm of the manometer to the 900-mm. reference mark. Record the height of the mercury column in the right arm as the zero reading.

Now close stopcock 5 and, by means of stopcock 2, connect the bomb manifold to the system leading to the manometer, by-passing that volume between stopcocks 1 and 2. Open one of the Hoke valves on the bomb manifold and allow atmospheric pressure to enter the system. Close the Hoke valve and again adjust the mercury column to the reference mark. Record the reading in the right arm as sample reading.

After lowering the mercury in the manometer as previously instructed, open stopcock 5 leading to the evacuated 1000-ml. flask and allow the system to come to equilibrium. Measure the pressure in the usual manner and record as equalized pressure reading.

From these data determine r_{1000}, the ratio of the 1000-ml. flask to the unit volume, as follows:

$$r_{1000} = \frac{\text{sample reading} - \text{equalized pressure reading}}{\text{equalized pressure reading} - \text{zero reading}} \quad (1)$$

In a similar manner determine r_{2000} and r_{3000}.

In addition to these three ratios a fourth, k_{500}, must be determined. This is defined as:

$$k_{500} = \frac{\text{volume of 500-ml. flask}}{\text{volume of apparatus manifold}} \quad (2)$$

Determine k_{500} directly in the above-described manner, in the special case using the manifold alone in place of the unit volume and measuring the pressure drop when the 500-ml. flask is turned into the system. The use of k_{500} will be apparent later.

Calculation of *R* Values. Since it is obviously impossible to use any flask or combination of flasks without employing the use of the manifold volume, the measured ratios (r_{1000}, r_{2000}, r_{3000}, and k_{500}) cannot be used per se but serve as data for calculating the ratio of any flask combination plus manifold to the unit volume.

These latter ratios are referred to as *R* values. The *R* value once determined for any given instrument, serve as a basis for computation with any subsequent sample prepared using the apparatus.

It is obvious from Equation 2 that for a single flask, *a*,

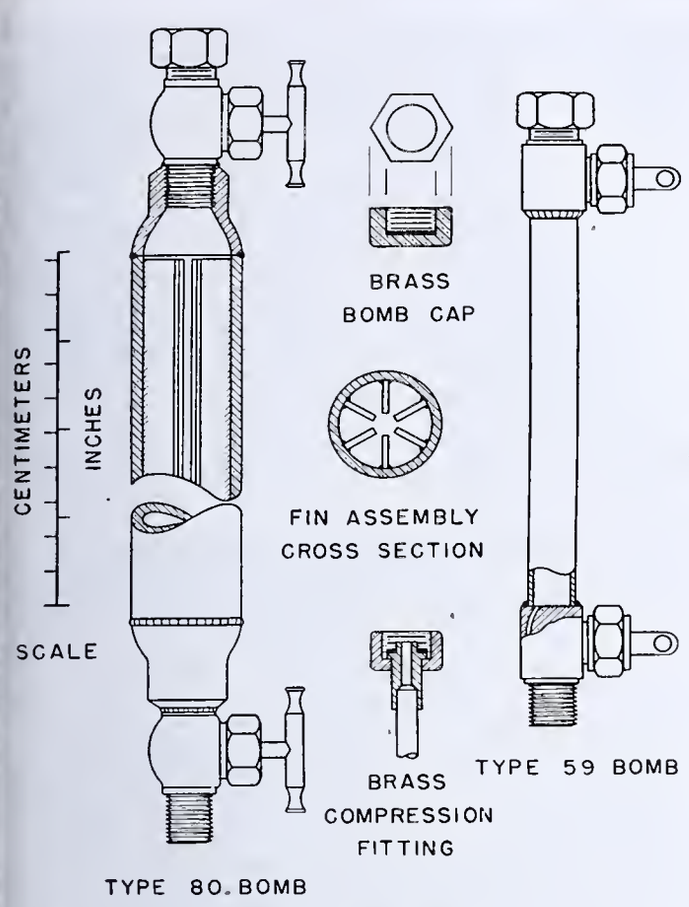

Figure 3. Bomb Detail

$$R_a = \frac{V_a + V_m}{\text{unit volume}} = r_a + \frac{V_m}{V_m + V_m k_{500}} = r_a + \frac{1}{k_{500} + 1} \quad (3)$$

Likewise for any combination of flasks

$$R_{(a\,+\,b\,+\,\ldots x)} = r_a + r_b + \ldots r_x + \frac{1}{k_{500} + 1} \quad (4)$$

For those flask combinations involving the use of the 500-ml. lask, the following simplified relationship may be derived

$$R_{(a\,+\,500)} = \frac{V_a + V_{500} + V_m}{\text{unit volume}} = r_a + 1 \quad (5)$$

Occasionally it has proved necessary to prepare a sample containing only a trace of one component. On these occasions it is lesirable to use the volume of the manifold alone. Consequently, R_m must be calculated.

$$R_m = \frac{V_m}{V_{500} + V_m} = \frac{1}{k_{500} + 1} \quad (6)$$

Using Equations 3 to 6, calculate the ratios for all flask combinations and for the manifold alone, tabulating the various ratios in suitable form.

Volume of the Apparatus. The number of entries of a given component into the apparatus is determined by the volume of the flask combination used, the sample size, and the percentage of that component desired in the finished sample. At this stage, we are primarily interested in the first of these considerations, the volume of any given flask combination. Since the R values calculated above relate all combinations to the unit volume, it is necessary to determine only the latter.

The volume is obtained by entering any pure hydrocarbon from a small weighed copper bomb into the unit volume of the apparatus. The pressure of the gas is measured and using the loss in weight of the small bomb, the approximate volume calculated. By reference to the R values, the volume of any flask composition may readily be obtained.

SAMPLE PREPARATION

Preliminary Calculations. Assuming that the total weight and composition of the desired sample have been established, it is now necessary to determine the flask combination and the approximate pressures to be used for each individual component. As an actual example, the calculations involved in the preparation of a butane-pentane sample are presented.

Letting a represent the mole fraction of component A in the finished sample and V_s the total sample volume desired (liquid ml.), it can be calculated that the approximate gaseous volume (760 mm. and 27° C.) to be added if A is C_4 material is

$$\text{Volume } (C_4 \text{ material}) = 254\ V_s a \quad (7)$$

Similarly, if A is C_5 material

$$\text{Volume } (C_5 \text{ material}) = 205\ V_s a \quad (8)$$

The volume calculated by Equation 7 or 8 is now divided by the capacity of the unit volume, obtained in the preceding calibration. The number so obtained represents the number of unit volumes which must be introduced into the apparatus at 760-mm. pressure to obtain the desired amount of component A.

At this point, however, deviation from the ideal gas law must be considered. It can be calculated that, should C_4 and C_5 materials be entered at 760-mm. pressure, the deviation of the former from ideality would amount to approximately 3.1% while the deviation of C_5 material would be 4.6%. This is obviously too high a differential for accurate preparation. Consequently, it has been the practice in this laboratory to enter the C_4 materials at 300-mm. and the C_5 materials at 150-mm. pressure. Under these conditions the successive entries of a component may be kept to a reasonable number and the deviations from ideality safely disregarded.

Multiplication by the appropriate pressure factor yields values which may now be compared directly with the R values obtained in the calibration procedure. The flask combination having an R number or multiple thereof approach most closely the calculated value is chosen as that to be used for the component under consideration. (These directions assume that each entry will be made at 300-mm. for C_4 materials and 150-mm. pressure for C_5 materials. If preparation to an exact predetermined percentage is desired, the pressure of each entry must be modified accordingly. The conversion factor is simply the ratio of the desired and actual R values.) It is obviously desirable to keep the number of entries to a minimum and the pressure of a single entry to a maximum (not over 300 mm. for C_4 materials, nor 150 mm. for C_5 materials).

The above calculations are repeated for each component in the sample.

Filling and Weighing Copper Bombs. If only 5 to 6 grams of hydrocarbons are required for a single component, a small copper bomb (Type 59) may be used; otherwise it will be necessary to use the larger type (Type 65). (The smaller bomb may be weighed with an ordinary analytical balance but the large bombs require the use of a balance of high capacity. A series of small bombs may be used, however, if large volumes are necessary and a high-capacity balance is not available.)

Connect the copper bomb to a large stock bomb of pure hydrocarbon, attach a vacuum pump, and evacuate the system to the closed valve of the stock bomb. Close the valve next to the pump, disconnect the pump, and cool the copper bomb by pouring liquid nitrogen over its surface from one Dewar flask to another. Now hold the stock bomb in an inverted position such that when its valve is opened the liquid phase of the contained hydrocarbon will flow into the cooled bomb. Open the valve on the stock bomb and, after a suitable interval, close the valve on the small bomb. Tilt the system in such a way that excess liquid hydrocarbon may drain from the connecting link into the stock bomb. Close the valve on the stock bomb and disconnect. Hold the small bomb in a vertical position and vent approximately 0.5 gram of the hydrocarbon from the bottom valve. (When a liquid-full bomb is warmed it becomes slightly distorted in shape.) Allow the bomb to warm to room temperature. No hazard is involved, since liquid-full copper bombs stretch without rupturing on warming to room temperature.

For hydrocarbons normally liquid under atmospheric conditions, the following technique is used.

Connect the evacuated copper bomb to a funnel by means of a clean piece of neoprene tubing. Support funnel and bomb in a vertical position and pour into the funnel the desired amount of liquid hydrocarbon. Open the upper valve of the bomb slightly and cool the bomb with liquid nitrogen until all hydrocarbon passes into the bomb. Close the valve, disconnect the funnel, vent approximately 0.5 gram of material, and allow the bomb to warm to room temperature. After washing with acetone and drying, accurately weigh each filled bomb on an analytical balance.

Testing the Apparatus. Mount the weighed bombs filled with hydrocarbon on the bomb manifold, making sure that the neoprene gaskets in the manifold connections are in good condi-

Table I. Typical Samples

Sample No.	Component	Pressure Basis *Mole %*	Weight Basis *Mole %*
1	2-Methylpropane	19.89	19.85
	2-Methylpropene	9.80	9.80
	1-Butene	10.53	10.52
	2-Butene	20.21	20.25
	n-Butane	39.57	39.58
2	2-Methylpropane	69.86	69.88
	2-Methylpropene	5.03	5.05
	1-Butene	5.00	4.98
	2-Butene	5.08	5.08
	n-Butane	15.03	15.01
3	2-Methylpropene	2.22	2.24
	2-Butene	11.33	11.34
	2-Methylbutane	59.20	59.21
	1-Pentene	19.52	19.49
	n-Pentane	7.73	7.72
4	2-Methylpropene	2.41	2.44
	2-Butene	11.98	12.09
	2-Methylbutane	57.12	57.03
	1-Pentene	20.23	20.23
	n-Pentane	8.27	8.22
5	2-Methylpropane	3.26	3.21
	1-Butene	1.71	1.68
	2-Butene	2.40	2.38
	n-Butane	8.55	8.48
	3-Methyl-1-butene	4.16	4.12
	2-Methylbutane	57.43	57.80
	1-Pentene	16.26	16.12
	n-Pentane	4.86	4.84
	2-Pentene	0.69	0.69
	2-Methyl-2-butene	0.68	0.69

tion. Connect the weighed sample bomb (with a lead-lined cap on the lower end) and the freezing-out bomb to the appropriate connection. Fill the water bath and regrease all stopcocks which turn with difficulty or appear streaked. Adjust the mercury columns in the manometer as previously described and evacuate the entire system including the sample bomb, freezing-out bomb, expansion flasks, and manifold. Open the Hoke valves on the manifold below the bombs, permitting evacuation to the bombs containing the hydrocarbons.

With the system evacuated, close off the expansion flasks by means of stopcocks 4, 5, 6, and 7. Place the system "on test" by closing stopcock 1, and observe the manometer. Eliminate any leaks by isolating critical parts of the system.

When the mercury remains steady, turn stopcock 8 to connect the sample bomb directly to the manometer. Turn the heater switch to "on" and cool the sample bomb in liquid nitrogen.

If no leak is apparent after the bomb has completely cooled, the sample bomb with its connection is satisfactory for use. If a leak is observed, remove the bomb and reconnect it to the apparatus by means of the other valve. If a leak is still apparent, replace with another weighed sample bomb.

Introduction of Hydrocarbons. Enter first that hydrocarbon which will be present in the smallest amount in the finished sample. The next largest component is then entered, etc.; enter last that hydrocarbon present in the greatest amount in the finished sample. (This order of entry is followed in the preparation of samples containing C_4 and C_5 materials. It may prove desirable with lighter hydrocarbons to enter the components in the reverse order of their molecular weight, thus keeping the residual pressure after condensation to a minimum.)

Open to the system the expansion flask (or combination thereof) to be used for component *A*. Close all Hoke valves on the bomb manifold except the one beneath the bomb containing component *A*. Evacuate the system and record the zero reading in the usual manner. Turn stopcocks 3 and 8 to by-pass the bombs. Close stopcock 1 and turn stopcock 2 to connect the bomb manifold to the system leading to the manometer. Carefully open the valve on the bomb containing component A, allowing hydrocarbon to vaporize into the system until the appropriate pressure is obtained. Close the valve on the bomb. Adjust the mercury column in the left arm of the manometer to the 900-mm. reference mark and record the sample reading.

Withdraw the mercury from the manometer until the total length (sum of readings in both arms) does not exceed 950 mm. Turn stopcock 8 to connect all arms. Add liquid nitrogen to the Dewar flask surrounding the sample bomb if necessary. After the manometer indicates no further condensation, turn stopcock 8 to by-pass the sample bomb.

The procedure to be followed at this point depends upon the number of entries of this component which are to be made.

Case I. A Single Entry. Usually a small amount of hydrocarbon will not freeze into the sample bomb. The residual pressure is due to phase equilibria at the surface of the cold hydrocarbon in the sample bomb; it must be taken into consideration before accurate composition can be calculated on a weight basis.

Lower the mercury in the manometer as usual. Slowly raise a half-pint Dewar flask of liquid nitrogen around the freezing-out bulb, thereby condensing practically all residual hydrocarbon in the system. After condensation is complete as indicated by a steady manometer reading, close off all expansion flasks in use from the system. Lower the liquid nitrogen and replace it with a beaker of warm water, vaporizing the hydrocarbon into the small glass manifold. As soon as the manometer indicates a pressure in the manifold greater than the residual pressure of the sample bomb, open stopcock 8 to all arms and allow as much additional hydrocarbon as possible to freeze into the sample bomb. When the manometer again indicates equilibrium, by-pass the sample bomb with stopcock 8, open all expansion flasks previously used, and, after adjusting the manometer level to the reference mark, record the zero reading after freezing.

The small amount of hydrocarbon which yet remains in the system must be measured, in order that this correction may be applied to the loss in weight of the manifold bomb containing component *A*.

Slowly raise a small Dewar flask containing liquid nitrogen around the freezing-out bulb and place a similar flask about the liquid nitrogen traps. Turn stopcocks 2 and 3 to connect all arms. Turn on the vacuum pump and slowly open stopcock 1. All residual hydrocarbon will be caught in either the freezing-out bulb or the liquid nitrogen traps, while any inert material present in the system will be pumped out. It is essential to remove all traces of inert matter at this point, as it greatly interferes with the subsequent freezing-out of the last traces of hydrocarbon in the system. After the manometer indicates that evacuation is complete, close off allex pansion flasks in use and close stopcock 1.

Turn stopcock 2 to by-pass the bomb manifold. Remove the Dewar from around the liquid nitrogen traps and replace with a beaker of warm water. After all hydrocarbon has vaporized from the traps and condensed into the freezing-out bulb, turn stopcock 2 to connect the bomb manifold and the liquid nitrogen traps, thereby isolating all hydrocarbon in the small glass manifold leading to the manometer. (Because of vacuum inside the traps, heat is transferred slowly to the inner tubes containing the hydrocarbon. Be certain all material has vaporized from these tubes before turning stopcock 2.)

Remove the liquid nitrogen from around the freezing-out bulb and place it around the freezing-out bomb, covering only about an inch of the bomb above the lower valve. Place a beaker of warm water around the freezing-out bulb to vaporize hydrocarbon from the bulb to the bomb. When the transfer is complete, close the upper bomb valve and, after by-passing the bomb with stopcock 3, disconnect it. Warm the bomb to room temperature under tap water, wash both its surface and ends with acetone, and remove any residual acetone in the ends with a vacuum pump. Wipe with a clean cloth and weigh. Evacuate the contents with a vacuum pump and reweigh.

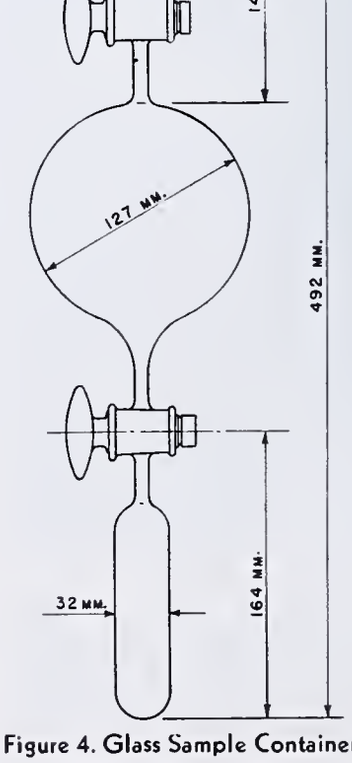

Figure 4. Glass Sample Container

Case II. More Than One Entry. Immediately after by-passing stopcock 8, adjust the mercury level to the reference mark, and record the zero reading after freezing.

Treat all subsequent entries except the last one exactly like the first entry, recording only the sample reading and zero reading after freezing for each entry. The residual pressure after condensation is not pumped out between successive entries of a single component. Make all corrections upon the last entry. Treat the last entry exactly as described in Case I.

Repeat the above procedure for each component comprising the sample, evacuating the system between the entries with the

mple bomb cut off from the system by means of stopcock 8. fter the last component has entered the sample bomb, close the lve on the latter, remove the liquid nitrogen surrounding it, d disconnect it from the apparatus. Warm to room temperare with tap water, wash with acetone in the usual manner, and igh.

Calculation of Sample Composition. *Pressure Basis.* The essure, p_1, of a single entry of component A with respect to a ven combination of expansion flasks is obtained by

$$p_1 = \text{sample reading} - \text{zero reading after freezing} \quad (9)$$

The total partial pressure, p_a, of component A with respect to at flask combination is simply the sum of the pressures of the dividual entries of that component.

$$p_a = p_1 + p_2 + \ldots + p_n \quad (10)$$

The total partial pressure, P_a, with respect to the unit volume calculated as

$$P_a = p_a R_a \quad (11)$$

ere R_a is that ratio listed in the table of ratios for the combinan of flasks used for this component.

The mole percentage, C_a, of component A in the finished sample then:

$$C_a = \frac{100\ P_a}{P_a + P_b + P_c \ldots + P_n} \quad (12)$$

here P_b, P_c, etc., are similarly calculated partial pressures of mponents B, C, etc.

Weight Basis. The total weight of an individual component leased into the system is equal to the loss in weight of the copper mb containing that component before and after sample preparation. The weight actually present in the sample, however, the above value minus that found in the freezing-out bomb ter removal of the residual hydrocarbon in the system.

Using the combined weight data, the mole percentage compoion is calculated in the usual manner.

TYPICAL SAMPLES

Table I illustrates the accuracy with which samples (50 liquid l.) in the butane and pentane range may be prepared. All itanes and butenes, as well as the *n*-pentane, isopentane, and 1-ntene, were research grade material purchased from Phillips etroleum Company.

COMMENTS

On the basis of the experience of this laboratory, it is estimated that a 50-liquid ml. sample containing six components can be prepared, obtaining both weight and pressure data, in 8 man-hours. Although this appears to be a considerable period, it must be remembered that a 50-ml. volume permits analysis of more than fifty infrared or mass spectrograph samples. Assuming that one check a day is made on these instruments, a single preparation would last approximately two months.

The time of preparation of large samples is greatly reduced if only pressure data are obtained. Approximately 4 man-hours are required to prepare a sample of any composition since, under these circumstances, weighings are unnecessary and the time necessary to freeze out the residual hydrocarbon of each component is saved.

The usual practice in this laboratory, when introducing an operator to these techniques, is to require him to prepare about four samples obtaining data which permit calculation of the composition on both a weight and pressure basis. At the end of this period enough experience has been acquired to obtain accurate results on a pressure basis alone.

Occasionally a "spot" sample of definite composition is required in conjunction with the infrared work. A small six-component mixture may be blended rapidly (less than 30 minutes), substituting the glass sample container shown in Figure 4 for the sample bomb. These samples are prepared on a pressure basis, using the glass manifold and the glass sample container (minus the cold finger) as the gas volume. Each component is frozen successively into the cold finger and any inert material removed with the pump exactly as described above.

Although the apparatus has been used only in conjunction with hydrocarbons, it is obviously applicable to the preparation of any sample whose components are liquefiable with liquid nitrogen.

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Method for Calcium-Sequestering Value of Phosphate Water Conditioners

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COMPARATIVELY recently molecularly dehydrated sodium phosphates have received considerable attention in the fields f water conditioning and detergency because of their ability to equester calcium, magnesium, and other metal ions in soluble rm such that these ions are not precipitated by fatty acid and ther radicals. Since the use of these phosphate water conditioners depends primarily upon this ability, it is becoming increasingly important to have available a precise analytical ethod for its determination.

A number of investigators have studied the calcium-sequestering properties of polyphosphates, but few data are available hich may be used to ascertain the true worth of the analytical ethods employed.

Huber (*3*) and Watzel (*6*) used a "residual hardness method" in which the polyphosphate is added to water of known calcium ontent and the excess calcium determined by titration with tandard soap solution. Andress and Wüst (*1*) preferred to titrate directly with standard calcium chloride solution, the end oint being marked by the appearance of a white turbidity. They titrated 0.4-gram samples at pH 10.0 to 10.5 to a definite turbidity as measured with a Zeiss-Pulfrich photometer and pointed out that the calcium values so obtained decrease with increasing concentration and temperature, but increase with increasing pH. Chwala and Martina (*2*) also titrated directly with calcium chloride, confirming the effect of pH in a general way, and pointing out that sharper end points are obtained at higher temperatures.

Rudy, Schloesser, and Watzel (*5*) noted that in the direct titration method the pH of the polyphosphate solution is progressively lowered by calcium chloride addition and attempted to eliminate this factor by keeping the pH fairly constant by adding sodium hydroxide from time to time during the titration. They also measured the calcium sequestering power of polyphosphates in the presence of precipitating anions such as oxalate and fatty acid radicals.

The present paper offers a tested procedure for determining "calcium value" and presents data showing the effects of some of the more important variables. In the past, this property has been expressed in terms of grams of calcium sequestered by 1 gram of sample. It is now proposed that calcium value be expressed as grams of calcium per 100 grams of sample—that is,

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A photometric titration method for precisely determining the ability of a molecularly dehydrated sodium phosphate to sequester calcium is described, and it is proposed that results be expressed as grams of calcium sequestered per 100 grams of sample. The effect of pH on results is marked and varies with the type of sample analyzed. Ability to sequester calcium increases with decreasing concentration, but this is not marked enough to affect results using the recommended sample size. The influence of variables suggests that the procedure be rigidly standardized and that departures from the standard conditions be indicated in presenting results. The method has a limit of uncertainty of ±0.4 for sodium tripolyphosphate having an average "calcium value" of 10.4. Duplication of results is usually excellent.

the per cent calcium sequestered by the material on the basis of the original sample. Thus, a calcium value formerly written as 0.127 becomes 12.7 (grams of calcium per 100 grams of sample), providing a simpler and more readable number and affording a better basis for comparing different values.

REAGENTS AND APPARATUS

The only reagents required are approximately 0.1 *N* sodium hydroxide and standard calcium chloride solutions. The sodium hydroxide solution is prepared carbonate-free without the addition of barium hydroxide; this is conveniently done by diluting a clarified 50% solution of analytical grade sodium hydroxide with boiled distilled water. The standard calcium chloride solution is prepared by dissolving 7.00 grams of pure calcium chloride (Baker's C.P. anhydrous, lump, for reagent use) in 1 liter of distilled water to give a neutral solution containing about 0.25 gram of calcium per 100 ml. The calcium chloride solution is conveniently standardized by the well-known precipitation of calcium oxalate and subsequent titration with potassium permanganate. However, use of the specified grade of calcium chloride permits standardization by simply titrating the chloride present: Duplicate 25-ml. aliquots are titrated in the presence of 1 ml. of 5% aqueous potassium chromate to a salmon end point with standard 0.1 *N* silver nitrate. Two solutions analyzing 0.252 and 0.236 gram of calcium per 100 ml. by the oxalate-permanganate method gave values of 0.251 and 0.236, respectively, by the silver nitrate titration method.

A filter photometer employing a 40 × 40 × 60 mm. optical cell is used to locate the end point of the calcium chloride titration. An Eimer and Amend photoelectric colorimeter, consisting of a pair of electrically opposed barrier layer type photocells whose balance is ascertained by means of a Wheatstone bridge, was used in the present work. This particular instrument is no longer on the market, but any fairly stable spectro- or filter photometer measuring light transmittance through a cell of approximately the indicated dimensions should be equally satisfactory, provided there is enough space available to position a stirrer and titrating buret over the cell. Also necessary is a glass electrode pH meter to adjust the pH of the sample solution before titration. The Beckman pH meter, Laboratory Model G, was used throughout the present work. The assembly of apparatus is completed by a motor-driven stirrer consisting simply of a 15-cm. length of 6-mm. Pyrex glass rod slightly flattened at the lower end, a 50-ml. buret with offset delivery tube, and a square yard of light weight black cloth.

PROCEDURE

Dissolve a 4.000-gram sample and dilute to exactly 250 ml. with distilled water. After thorough mixing, if the solution is turbid, allow the insoluble matter to settle out or filter through a dry No. 42 Whatman paper into a dry vessel. Pipet 25 ml. of the clear solution into the 40 × 40 × 60 mm. optical cell, dilute to about 45 ml., and adjust the pH to 10.0 by dropwise addition of 0.1 *N* sodium hydroxide, using the pH meter and ordinary glass electrode and omitting the correction for sodium-ion concentration. After rinsing the electrode assembly into the optical cell, dilute the solution to about 55 ml., conveniently determined by previously calibrating the cell. Place the optical cell in the photoelectric colorimeter and insert the glass stirrer in the right-hand corner of the cell nearest the light source. Place the titrating buret in position and completely hood the optical cell and adjacent sections of the apparatus with the black cloth. Adjust the colorimeter to read 100% transmittance with the stirrer on using Wratten filter H No. 45 in the Eimer and Amend instrument, or any available deep blue filter in a similar instrument.

Titrate with the standard calcium chloride solution until a sharp decrease in transmittance is obtained, taking at least three readings at 0.5-ml. intervals after the end point. For samples having calcium values of 9.0 or higher, it is advantageous to add about 14 ml. of titrant before making a reading. Readings at 1 ml. intervals should then be taken until the end point is reached when the intervals should be cut to 0.5 ml.

The calcium value of the sample, expressed as grams of calcium sequestered by 100 grams of sample, is obtained by plotting the observed transmittance readings against the corresponding milliliters of titrant added and drawing the best straight lines through the points before and after the break. The intersection of these lines gives the milliliters of calcium chloride required by the following calculation:

$$\text{Calcium value} = (\text{ml. of } CaCl_2)(\text{grams of Ca/100 ml.})(2.5$$

Often during a titration small changes in transmittance will occur due to fluctuations in line voltage, etc. If such a change is followed by essentially constant readings, it should be attributed to a change in the 100% transmittance setting and the prior readings disregarded in drawing the straight line through the plotted data.

EXPERIMENTAL WORK

Development of Method. The direct titration with standard calcium chloride was chosen as representing the simplest and most rapid method for determining calcium value. Before the procedure described above had been developed, and following the general technique of previous investigators, 0.4-gram polyphosphate samples dissolved in 25 ml. of distilled water and adjusted to pH 10.1 to 10.3 were titrated with 0.06 *M* calcium chloride to the first appearance of a turbidity. The end point was observed visually, a flashlight beam being directed through the titration cell to increase sensitivity. Rather extensive testing of the precision of this procedure, using different analysts, indicated that while fair duplication of results was usually obtained at any given time, visual location of the end point is unreliable because of inability of the analyst to duplicate his previous day's value. The situation is also complicated by the occasional appearance of a slight turbidity before the true end point and by the varying behavior of different types of samples. Accordingly, it was considered advisable to locate the end point photometrically if a precise method were to be developed.

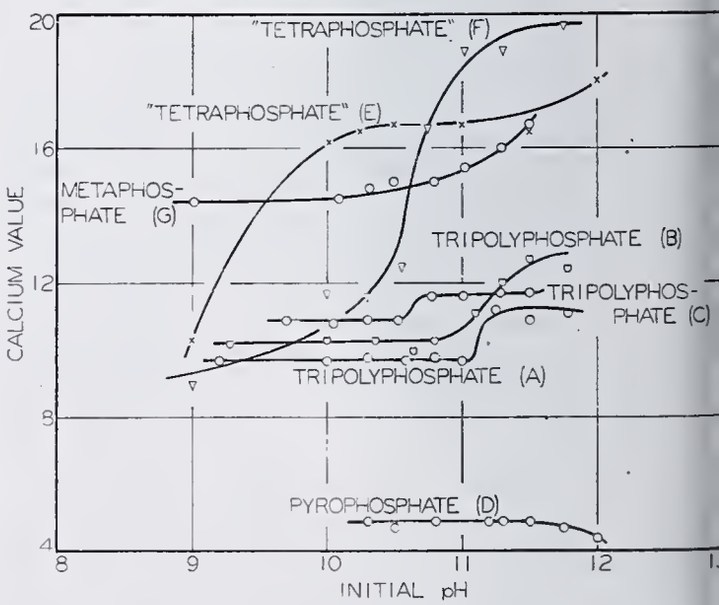

Figure 1. Effect of Initial pH on Calcium Values of Various Polyphosphates

Photometric titrations on different samples were carried out dissolving 0.4 gram of sample in 50 ml. of distilled water and usting the pH to 10.0. This solution was placed in the 40 × × 60 mm. optical cell in the path of the light beam in the Eimer l Amend colorimeter. A motor-driven stirrer, consisting of a ight glass rod flattened at the lower end, was inserted in the ution, and the light transmittance adjusted to read 100%. ndard calcium chloride solution was then added, the transmit- ce being determined at intervals during titration.

The results obtained made it clear that in the majority of cases transmittance remains at or near 100% until the end point is ched and then decreases linearly with added titrant. This bles the analyst to draw straight lines through the plotted nts after the break as well as through those before the break, l to take their intersection as the end point. This procedure uld appear to be more valid than titrating to a definite measur- e turbidity, which is obviously beyond the true end point. rthermore, the straight lines drawn through the plotted points er the break have been found to have differing slopes, indicat- that titrating to any given turbidity would give values vary- by different amounts from the true end point.

The choice of light filter to use in the colorimeter should not af- the determination greatly, since the colorimeter is used only find the end point. However, a number of Wratten gelatin ers were tested, and filters H No. 45 and C5 No. 47, both nsmitting deep blue light, were found to give the greatest nge in transmittance for a given unit of turbidity. Filter H . 45 was arbitrarily chosen for the present method. Use of a p blue filter has the advantage that in the graphical calcula- n involved, the intersecting lines are more nearly at right angles n if a less sensitive filter were used.

t was thought that the position of the glass stirrer in the opti- cell might have some effect on the results obtained, since the rer must be placed at least partially in the light beam because the dimensions involved. A few tests indicated that small nges in position do not affect the relativity of the transmit- ce readings, and if the stirrer is left in the same position during letermination, no interference is encountered. It was dis- ered, however, that extraneous light might cause considerable or, especially when light conditions are changing. To nullify this effect the optical cell and adjacent portions of the apparatus were shrouded with black cloth.

The effects of titration time and time lapse between calcium chloride addition and transmittance measurement were also tested. In the one case, varying the time required to complete the titration from 2 to 8 minutes gave four calcium values of 11.0 for a sample of sodium tripolyphosphate. In the other, varying the time interval between calcium chloride addition and transmittance measurement from 10 to 60 seconds gave values having a total calcium value range of 0.2. Small changes in temperature were also found to be relatively unimportant. A series of titrations in which the initial temperature varied from 20° to 30° C. gave calcium values ranging from 11.1 to 10.9 for the above sample of sodium tripolyphosphate.

Effect of pH. The previously cited work of other investigators and early unpublished data obtained in this laboratory by the visual end point calcium value titration method had definitely shown that pH is an important variable in the determination of calcium value.

In order to determine the effect of pH on the present method, the calcium values of various types of polyphosphates were determined after adjustment of the sample solutions to different pH levels. Also, since pH had been found to decrease during a titration with calcium chloride, the end point pH values were determined on separate aliquots to which the amount of titrant required to reach the end point had been added. Three samples of commercial sodium tripolyphosphate, two samples of commercial sodium "tetraphosphate", one sample of commercial anhydrous tetrasodium pyrophosphate, and one sample of glassy sodium metaphosphate were tested as being representative of the types of material employed in water conditioning. The metaphosphate was prepared by fusing about 25-gram lots of C.P. monosodium orthophosphate in a platinum crucible at 700° to 800° C. for one hour and quenching the hot crucible in ice water after rotating to obtain a thin film of melt on its inside surface. The results obtained are shown in Table I, and the initial pH values are plotted against calcium value in Figure 1. Uncorrected pH values obtained with a glass electrode are used throughout.

Inspection of Figure 1 reveals that determined calcium value is affected by the pH to which the sample solution is initially adjusted in a marked and variable manner. For sodium tripolyphosphate, the calcium value appears to be fairly constant up to a pH of 10.5 to 11.5, in which range a reasonably sharp increase in calcium value occurs. The break does not seem to be limited to a very narrow pH range. The sodium "tetraphosphate" samples both show remarkable increases in calcium value at rather different pH levels. The calcium values of the tetrasodium pyrophosphate and glassy metaphosphate samples are more constant, the latter showing a fairly slow increase with increasing pH.

These findings indicate that for sodium tripolyphosphate, at least, the procedure of adjusting to pH 10.0 gives calcium values in a pH region where pH has little effect. The flat portions of the calcium value–pH curves for tripolyphosphate are not in agreement with results obtained by titrating to the first visual appearance of a turbidity, or by titrating to a given instrumental turbidity. The latter procedures usually give a steady increase in calcium value with increasing pH, and the present flat curves are probably the result of the improved method of locating the end point. The variability of the calcium value–pH data shown illustrates that use of the initial pH value of 10.0 is more or

Table I. Effect of pH on Calcium Values of Various Polyphosphates

Sample	Calcium Value	pH Initial	pH Final	Sample	Calcium Value	pH Initial	pH Final
ium tripolyphos- hate, sample A	9.7	9.21	8.30	Sodium "tetra- phosphate", sample E	10.3	8.99	8.00
	9.7	10.00	9.40		16.2	10.02	8.45
	9.8	10.30	9.80		16.5	10.25	8.65
	9.7	10.58	10.22		16.7	10.50	9.15
	9.8	10.79	10.55		16.7	10.75	9.90
	9.7	11.00	10.80		16.7	11.00	10.41
	11.2	11.25	11.01		16.5	11.50	11.35
	10.9	11.50	11.32		18.0	12.00	11.99
	11.1	11.77	11.60				
ium tripolyphos- hate, sample B	10.2	9.28	8.40	Sodium "tetra- phosphate", sample F	9.8	7.51	6.39
	10.3	10.00	9.50		9.0	9.01	8.42
	10.3	10.36	10.00		11.7	10.00	9.25
	10.0	10.64	10.42		11.6	10.29	9.55
	10.3	10.79	10.60		12.5	10.56	9.78
	11.1	11.10	10.90		16.6	10.75	9.20
	12.0	11.30	11.11		18.9	11.02	9.81
	12.7	11.50	11.35		18.9	11.31	10.92
	12.4	11.78	11.62		19.6	11.75	11.58
ium tripolyphos- hate, sample C	10.9	9.70	8.81	Sodium meta- phosphate glass, sample G	14.3	5.68	4.25
	10.8	10.05	9.22		14.4	9.02	7.25
	10.9	10.30	9.87		14.5	10.09	7.80
	10.9	10.53	10.19		14.8	10.32	7.92
	11.6	10.78	10.49		15.0	10.50	8.21
	11.6	11.01	10.70		15.0	10.79	8.30
	11.7	11.28	11.05		15.4	11.02	9.35
	11.7	11.50	11.22		16.0	11.29	9.50
					16.7	11.50	9.50
rasodium pyro- hosphate, sam- le D	4.9	10.30	10.19				
	4.7	10.50	10.39				
	4.9	10.80	10.71				
	4.9	11.19	11.11				
	4.9	11.30	11.22				
	4.9	11.50	11.45				
	4.7	11.75	11.71				
	4.4	12.00	11.99				

less arbitrary, and that no given initial pH will result in calcium values adequately comparing various types of polyphosphates. The calcium value of a given polyphosphate obviously has little meaning unless accompanied by a statement of the pH at which it was determined. In cases where the calcium value–pH behavior is unknown, maximum information is gleaned only from a calcium value–pH curve of the type shown in Figure 1.

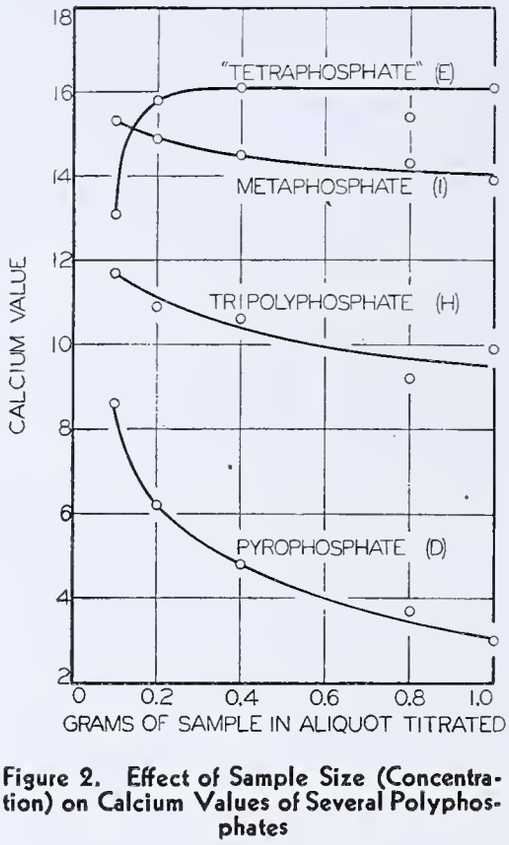

Figure 2. Effect of Sample Size (Concentration) on Calcium Values of Several Polyphosphates

During the development of the present method for calcium value, a few determinations were made using a calcium chloride solution that had been adjusted to pH 10.0 in an attempt to eliminate the difference between "initial" and "end point" pH values. However, owing to the difficulty of maintaining the unbuffered reagent at pH 10.0 and because the pH was not constant during titration in any case, it was decided to eliminate this feature in the interest of simplicity.

Effect of Sample Size. Andress and Wüst (*1*) have shown that the calcium value of sodium tripolyphosphate decreases with increasing concentration in the range 3 to 35 grams per liter, the greatest decrease occurring at about 10 to 15 grams per liter. Chwala and Martina (*2*) observed the same general effect for various polyphosphates. The effect of varying sample size on the present method was determined for commercial sodium tripolyphosphate, sodium "tetraphosphate", tetrasodium pyrophosphate, and laboratory prepared glassy sodium metaphosphate. The pyrophosphate and one of the "tetraphosphate" samples used in the pH study were employed. A different laboratory batch of glassy metaphosphate and a different sample of commercial tripolyphosphate were used. The calcium values obtained for different sample sizes of these materials by the present method are shown in Table II and plotted against sample size in Figure 2. Since the method specifies a volume of about 55 ml. at the start of the titration and since about 15 to 20 ml. of titrant may be added, the final concentration using a 0.4-gram sample corresponds to about 5.5 grams per liter in the method as written.

Figure 2 confirms the general effect of concentration on calcium value noted by other investigators. The curves are fairly flat at a sample size of 0.4 gram, indicating that sample size should not affect the precision of the method greatly. The steepest curve in this concentration range is found for tetrasodium pyrophosphate; a sample size difference of 0.1 gram in this case would give a difference in calcium value of about 0.5.

Precision. The precision of the present method for calcium value has been found to vary somewhat, depending on the type of sample being examined. In order to test precision, ten determinations were made by a single experienced analyst on a sample of sodium tripolyphosphate. Treatment of the data (shown in Table III) as outlined by Moran (*4*) gives an LU_1 of ± 0.4 against an average calcium value of 10.4. LU_1 is the symbol for the limit of uncertainty of the method under the best conditions. For data which are statistically normal, this LU_1 implies that a single determination has 997 chances out of 1000 of being within 0.4 unit of the average calcium value.

On many samples, duplicate determinations check somewhat better than might be indicated by the above precision. For instance, in determining the calcium values of various polyphosphate mixtures, eight sets of duplicate determinations gave an average difference between duplicates of only 0.05. In other cases, where the end point is not so well defined, duplicate determinations may vary by as much as 0.4. In general, the method has been found capable of precise results on most of the materials tested.

CONCLUSIONS

The calcium-sequestering ability of sodium polyphosphate water conditioners can be determined with good precision by titration with standard calcium chloride solution, using a filter photometer and graphical computation to ascertain the point at which a precipitate first begins to form. Results should be expressed as grams of calcium sequestered by 100 grams of sample. Increasing concentration decreases calcium value, but small differences in sample size do not greatly influence the precision of the method. Calcium values of different polyphosphates are affected in a marked and variable manner by the pH level to which the sample solution is adjusted before titrating. The precision of the method, in terms of limit of uncertainty under the best conditions, was found to be ± 0.4 for sodium tripolyphosphate having a calcium value of 10.4. Precision varies, depending on the type of sample and conditions of determination.

Since the calcium value of phosphate water conditioners varies

Table II. Effect of Sample Size on Calcium Values of Various Polyphosphates

Sample	Calcium Value				
Sample size, grams	0.100	0.200	0.400	0.800	1.00
Approximate concentration, grams per liter	1.4	2.8	5.5	11.0	13.8
Sodium tripolyphosphate sample H	11.7	10.9	10.6	9.2	9.9
Tetrasodium pyrophosphate, sample D	8.6	6.2	4.8	3.7	3.0
Sodium "tetraphosphate", sample E	13.1	15.8	16.1	15.4	16.1
Sodium metaphosphate glass, sample I	15.3	14.9	14.5	14.3	13.9

Table III. Precision of Method for Calcium Value

Calcium Value	Deviation	(Deviation)^2
10.27	0.17	0.0289
10.32	0.12	0.0144
10.39	0.05	0.0025
10.32	0.10	0.0100
10.59	0.15	0.0225
10.49	0.05	0.0025
10.49	0.05	0.0025
10.52	0.08	0.0064
10.61	0.17	0.0289
10.39	0 05	0.0025
Average		10.44
Average deviation		0.10
Standard deviation, σ		0.11
LU_1, $3\sigma/0.923$		± 0.36
Range, 10.61–10.27		0.34

th such factors as pH and concentration, the conditions of the termination must be rigidly standardized, and any departure om these conditions should be noted in expressing results. The lcium value obtained may have little connection with water-ftening power under conditions of use other than those of the ethod. Where performance under conditions of actual use is quired, the method may be modified, but only with the under-anding that the resulting data cannot be compared directly th data obtained by using the method as written.

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Radioactive Studies

Analytical Procedure for Measurement of Radioactive Arsenic of 90-Day Half-Life[1]

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procedure for the quantitative radioactive analysis of arsenic 90-day half-life depends upon the isolation of arsenic metal by duction with hypophosphite, collection on a Selas filter plate, and easurement of the activity inside the chamber of a Lauritzen elec-oscope. The utilization of 90-day arsenic rather than the 16-day otope extends considerably the duration of experimentation fol-wing cyclotron bombardment.

URING the course of investigations utilizing 16-day As^{74} as a tracer, it was found that an appreciable amount of radio-tivity due to 90-day arsenic is also present. This long-lived senic (*2*) is known to be either As^{72} or As^{73}. It disintegrates to $Ge^{72, 73}$ by the capture of a K-shell electron and the emission a gamma-ray of about 0.05 Mev. Through photon absorption, is gamma-ray may result in the ejection of either a K- or L-shell ectron surrounding the germanium nucleus. Such electrons are own as internal conversion electrons and in the case of 90-day senic the process is highly efficient. A special procedure has en worked out for the detection of these low energy (approxi-ately 0.05 Mev.) electrons.

Both the 16- and 90-day arsenic isotopes are formed by the euteron bombardment of germanium. On the basis of a 10-ur bombardment by the M.I.T. cyclotron of Ge and the addi-n of 50 mg. of carrier As^{75} for separation purposes, it was pos-ble with As^{74} to detect 0.005 microgram and make quantitative dioactive analyses to a 0.1 microgram of arsenic for a period of ly 10 to 12 weeks. The procedure described for the detection the 90-day isotope produced by the same bombardment ex-nded these analyses to a period of about one year.

DETECTION APPARATUS

Although the usual thin-walled cylindrical Geiger counter (*1*) the thin mica window helium-filled bell-type counter (*5*) is tisfactory for detection of the 16-day isotope, it is not satisfac-ry for detection of the internal conversion electron emitted by e 90-day isotope. This is due to the large fraction of these soft ectrons that are absorbed by either the window or wall of the ove counters. Since it has not as yet proved feasible to make utine radioactive analysis to better than 15% accuracy by acing samples inside counters, recourse is taken to the use of e modified Lauritzen electroscope (*4*).

As in the case of S^{35}, the arsenic samples are placed inside the electroscope chamber. In view of the low energy, nearly all the ionization resulting from inelastic collision of the electron with air molecules takes place in the chamber. This is the reason for the much greater sensitivity of the modified electroscope over the thin mica window counters in this particular case. The situation is reversed with the 16-day arsenic isotope, because of the high energy of the emitted particles. Thus, during the early period following the cyclotron bombardment, the concentration of the 16-day isotope is relatively high and the Geiger counter has a greater sensitivity as compared to background than the modified electroscope. This is of little import, however, since during this interval the specific radioactivity is also high.

ANALYTICAL METHOD

The radioactive determination is made on finely divided arsenic metal. Arsenic in either the +3 or +5 state of oxidation is quantitatively reduced to arsenic metal by alkali hypophosphites in strong hydrochloric acid solutions. The procedure on a micro scale was developed by Polyakov and Kolokolov (*3*). One procedure for obtaining the arsenic initially in the form of arsenate, which is well suited for biological investigations, is to oxidize the sample containing the arsenic by the Carius method. Sufficient nonradioactive (carrier) arsenic is added in the form of $H_2AsO_4^-$ to produce 4 mg. as arsenic metal.

The Carius solution containing 4.0 mg. of arsenic as $H_2AsO_4^-$ is transferred to a 25-cc. beaker and evaporated to dryness to remove the nitric acid. The residue is taken up in 3 cc. of water and 0.1 to 0.2 gram of sodium hypophosphite is added, followed by about 10 to 15 cc. of 7 *N* hydrochloric acid. The solution is then heated on a hot plate until the clearing of the supernatant layer (30 to 45 minutes) signifies that the precipitation is complete.

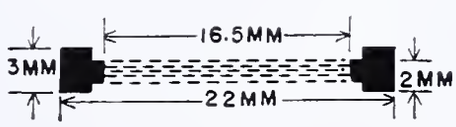

Figure 1. Cross-Sections of Filter Crucibles Prepared from Selas Crucible 2001

Effective filtering area, 2.15 sq. cm.

The arsenic precipitate is collected in a manner similar to that used for benzidine sulfate in the radioactive sulfur work (*4*), except that a Selas filter disk (Selas Co., Philadelphia, Pa.) is substituted for the filter paper. The finely divided state of the arsenic metal necessitates the use of these filter disks, which are prepared by cutting off the top of a Selas filter crucible No. 2001. After the filtering areas are immersed in paraffin for protective purposes, the disks are ground to identical size as indicated in Figure 1. The paraffin is then removed with hot ligroin.

[1] Second article on subject, "Radioactive Studies". The first appeared page 349, June 15 issue. Succeeding papers appear on pages 417 and 0. The fifth paper will appear in a later issue.

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Table I. Radioactivity Measurements as a Function of 90-Day Arsenic Content of $H_2AsO_4^-$ Using Modified Lauritzen Electroscope

(Dilution experiments. Concentration of least active sample is taken as unity. Radioactivity measurements are expressed as ratio of net activity average background.)

Radioactive Arsenic Concentration, C_i	Radioactive Measurements Observed, R_i	Expected[a], R_i	Average Deviation, %
250	181	181	0.3
	182		
	Av. 182		
200	145	145	0.0
	145		
	Av. 145		
125	90.2	90.6	0.2
	90.6		
	Av. 90.4		
100	73.8	72.5	0.9
	72.5		
	Av. 73.2		
50	35.0	36.3	1.1
	36.8		
	Av. 35.9		
25	17.5	18.1	1.4
	18.2		
	Av. 17.9		
20	14.6	14.5	2.4
	15.1		
	Av. 14.9		
12.5	9.27	9.06	1.6
	9.14		
	Av. 9.21		
10	7.28	7.25	0.7
	7.12		
	Av. 7.20		
5	3.43	3.63	3.3
	3.59		
	Av. 3.51		
2.5	1.78	1.81	5.3
	1.65		
	Av. 1.72		
2	1.49	1.45	5.2
	1.56		
	Av. 1.53		
1	0.70	0.73	8.3
	0.64		
	Av. 0.67		

[a] $R_i = \frac{C_i\Sigma(R_i/C_i)\log C_i}{\Sigma \log C_i}$. This equation expresses the fact that t accuracy of measurement increases somewhat with concentration of rad activity.

After the arsenic metal is collected on these disks, it is well washed with a hot reducing solution of 1% sodium hypophosphite in 7 *N* hydrochloric acid followed by absolute ethyl alcohol. Until ready for measurement these disks are kept in a vacuum desiccator.

The radioactive determination is now made by placing the disk containing the arsenic precipitate inside the electroscope chamber by means of the sliding bar device previously described (*4*). After the sample is measured, the arsenic is removed by the reverse flow of hot nitric acid. Alkaline oxidizing agents are to be avoided because of their injurious action on the porous plate.

Since the very low energies of the emitted electrons result in considerable self-absorption, uniformity of the thickness of precipitate over the filter disk area is especially important in quantitative work. Before preparing the disks, the Selas crucibles are selected with this in mind; visual observation of uniformity of precipitate suffices and about 70% of these crucibles pass inspection.

Although the hypophosphite reduction is quantitative (*3*), it cannot be used for gravimetric analysis, since arsenic metal forms an oxide film in moist air. The resulting increase in weight is consistently less than 2% if the procedure described above is strictly followed. This variation in the weight results in a maximum uncertainty of only 0.4% in the radioactive measurements of arsenic precipitates weighing about 4.0 mg. or 1.86 mg. per sq. cm. (Computed from Equation 2.)

ACCURACY OF RADIOACTIVE ANALYTICAL PROCEDURE AS MEASURED BY LAURITZEN ELECTROSCOPE

The accuracy of the analytical procedure was evaluated by the progressive dilution of the radioactivity, through the addition of known amounts of inactive arsenate ion to an arsenate solution containing 90-day arsenic. Each arsenate solution that was subjected to analysis contained 0.0552 millimole of arsenic (4.0 mg. as arsenic metal). As in the case of S^{35} (*4*), the data are presented in the terms of the following ratio:

$$\frac{(d/t)_s - (d/t)_b}{(d/t)_b} \qquad (1)$$

where d is the distance traversed by the electroscope fiber in time t, s indicates the radioactive sample, b denotes background, and $(\overline{d/t})_b$ is the average background rate of fiber discharge. T usual distance traversed by the electroscope fiber during mea urements is 10 small divisions.

Duplicate experimental determinations are presented in Tab I. The concentration of the 90-day arsenic present in the lea active sample is arbitrarily taken as unity.

The data indicate that radioactive analysis to 2% can be ma on all arsenic samples containing activity greater than about times background by the procedure given above.

SELF-ABSORPTION OF ELECTRONS BY ARSENIC METAL PRECIPITAT

Unfortunately it is not possible to prepare, as was done f radioactive sulfur (*4*), a correction table for self-absorption radioarsenic that is nonvarient in time. This is due to the tim dependent ratio of 16-day arsenic to 90-day arsenic. This is n a serious limitation, since in most chemical and biological expe ments involving the use of radioarsenic, the weight of the met precipitate can be predetermined by the addition of carrier in t form of nonradioactive arsenate. If the weight of arsenic met precipitate cannot be predetermined, it is necessary to have hand standards of different known weights of precipitate co taining equal radioactivity; this activity must of course resu from the same bombardment as the radioarsenic being analyze

It is found that 4 to 10 mg. (1.86 to 4.65 mg. per sq. cm of arsenic give a uniform distribution of metal precipitate on Sel plate. This weight should be kept minimal in view of the hi self-absorption.

After about 100 days following cyclotron bombardment, t effective concentration of 16-day radioarsenic is small as compar to 90-day arsenic. Thus from this time on, it would be possib to use a weight correction table for self-absorption. Data f this table have been obtained but are not given here in deta To within 4%, these data follow the following equation (*4*):

$$I = \frac{K}{\alpha d}(1 - e^{-\alpha d})$$

I is the measured radioactivity, K is a constant, d is the thic ness of precipitate in mg. per sq. cm., and α is an exponential a sorption coefficient of numerical value equal to 0.79 sq. cm. p

ıg. of arsenic metal precipitate. Self-absorption is more than 0% complete for a precipitate heavier than 5 mg. per sq. cm. nd is equal to 22% for a precipitate of about 2 mg. per sq. cm.

ACKNOWLEDGMENT

The authors are indebted to J. W. Irvine of the Massachusetts nstitute of Technology for suggesting the use of the Selas filter-ıg disk.

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Analytical Method for Determination of Long-Life Carbon C^{14}

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A detailed quantitative procedure for the measurement of radioactivity due to long-life C^{14} incorporated into organic compounds is described and evaluated. The method is based on the insertion of carbon dioxide gas into a quartz ionization chamber attached to a Lauritzen electroscope. The assemblage of this detection apparatus is described in detail. The method is especially suitable to biological work, since it will measure 3×10^{-5} microcurie of C^{14} diluted with as much as 20 millimoles of ordinary carbon.

RADIOACTIVE carbon C^{14} of half-life greater than 1000 years is certainly the most valuable of all the radioactive elements for general tracer work. It is usually prepared by the neutron bombardment of the N^{14} present in ammonium nitrate. Radioactive carbon is present in the form of carbon monoxide and carbon dioxide and is ultimately collected as barium carbonate (*8*). Because of the low yield of the N^{14} (n, p) C^{14} reaction, the production of appreciable quantities of C^{14} of high specific activity by cyclotron bombardment is very costly. However, the utilization of the neutron sources which are now available through the construction of uranium piles will make C^{14} more plentiful.

Ammonium nitrate, subjected to neutron bombardment by the M.I.T. cyclotron staff, was the authors' source of long-life carbon.

Long-life carbon disintegrates into N^{14} by the emission of a beta-particle of maximum energy of about 0.14 Mev. (*8*). Ruben and Kamen (*8*) have outlined a procedure for the radioactive analysis of C^{14}, which depends upon the isolation and precipitation of the activity in the form of a carbonate precipitate. The precipitate in milligram quantities is then placed inside a screen-wall counter and a certain fraction of the number of disintegrations is recorded. This procedure is subject to two serious limitations: (1) Geiger counters which are repeatedly subjected to the operations necessary for the insertion of a sample inside the counter are erratic; hence accuracy better than 10 to 15% is not to be expected. (2) Because of the low energy of the emitted particles, self-absorption is an appreciable factor, especially in biological tracer work, where the weight of a carbonate precipitate resulting from the oxidation of 1 gram of tissue is usually in grams. In view of the expense involved in the production of C^{14} of high millicurie strength, this factor must be minimized.

The procedure described was developed with both detection sensitivity and over-all accuracy in mind.

DETECTION APPARATUS

The ideal solution to the problem would be the insertion of carbon dioxide containing C^{14} inside a Geiger counter; under these conditions at least 90% of the radioactive disintegrations would be counted. One would have, then, a routine analytical method of optimum sensitivity which is applicable to all long-life radiocarbon compounds. Unfortunately this gas is not suitable for proper Geiger counter operation (no measurable plateau and erratic counting rates), and in view of the importance of C^{14} detection, considerable research has been directed toward the solution of this problem.

Recently it has been found (*6*) that the addition of certain contaminating vapors to carbon dioxide markedly improves these counter characteristics, but, as yet, this method cannot be adopted for accurate routine analyses.

It is also possible to introduce the gaseous carbon dioxide into an ionization chamber and to measure the ionization produced by the collisions of the emitted beta-particles with carbon dioxide molecules by means of an auxiliary apparatus. It was decided to measure this ionization with a Lauritzen electroscope (*5*). In view of its sensitivity, simplicity of design, ruggedness, and accu-

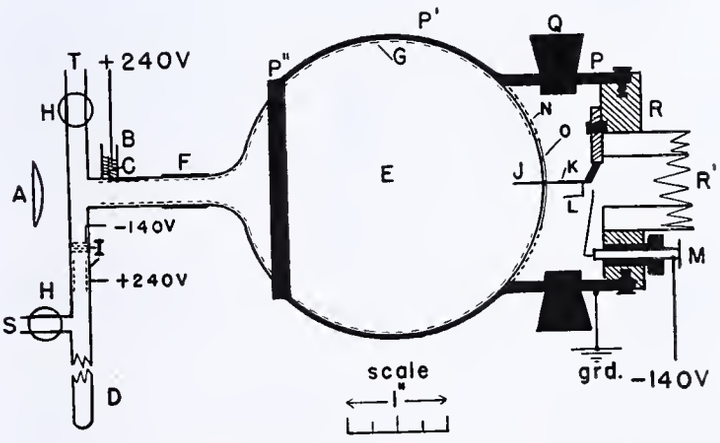

Figure 1. Cross Section of Ionization Chamber, Lauritzen Electroscope Detection Apparatus

A. Light source (115-volt 6-watt) for illumination of quartz fiber and scale
B. Mercury well
C. Tungsten wire providing electrical connection to silvered surface
D. Condensing tube
E. Quartz flask (ionization chamber)
F. Pyrex-quartz graded seal
G. Silvered portion of flask
H. High-vacuum pressure stopcocks
I. Ion trap (very coarse silvered fritted disk and silvered glass tube)
J. Ion collecting wire (3-mil tungsten)
K. Fiber-repelling post
L. Gold-plated quartz fiber
M. Electroscope charge button
N. Portion of flask coated with colloidal graphite (Aquadag)
O. Thin coat of ceresin wax
P. Brass adapter
P'. Brass straps, 0.25 inch (4 in all)
P''. Brass collar provided with screw for cinching straps
Q. Rubber-stoppered hole 0.375 inch, in brass adapter (4 in all)
R. Lauritzen electroscope frame
R'. Electroscope tube containing objective, scale, and eyepiece
S. To vacuum system
T. To mercury vapor pump

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racy, this electroscope is believed to be better suited for routine analytical work than (*9*) either a sensitive electrometer or a vacuum tube electrometer circuit.

The combination of the ionization chamber and Lauritzen electroscope is shown in Figure 1.

Assembling of Detection Apparatus. The neck of a 200-cc. quartz flask is drawn down and sealed to a quartz-Pyrex graded seal. An L-shaped tungsten lead wire, *C*, is tacked for a distance of 2 mm. along the inside wall of the Pyrex tube and then is sealed in. A minute hole (about 0.13 mm.), for subsequent introduction of the collecting wire, is made in the bottom of the flask, which is then sealed with a thin film of quartz.

After the inside surface is scrupulously cleaned, the interior spherical segment (about 3 cm. in diameter) which is to remain unsilvered is coated with vinyl acetate (Vinylite Resin AYAF, Carbide and Carbon Chemicals Corp., New York, N. Y.). This is done by clamping the flask in a vertical position and then introducing a 3% solution of vinylite in acetone to the requisite depth by means of a long capillary funnel. The excess vinylite solution is removed via the capillary funnel by suction. The remaining acetone is allowed to dry; this leaves a thin protective coating of vinylite over the small spherical segment.

The flask is evacuated with a water pump, and Brashear's silvering solution (*9*) is introduced by means of a stopcock. Removal of the silvering solution and subsequent rinsings are facilitated by use of the water pump. After the silvered surface is well washed with distilled water, the vinylite is dissolved with reagent acetone. Anhydrous reagent ether is used for final rinsing. The silvered surface is now tested for electrical continuity.

After the remainder of the glass apparatus shown in Figure 1 is attached, the outside lower half of the flask is well cleaned and dried. The thin quartz film sealing the hole in the bottom of the flask is pierced by means of a 5-mil tungsten wire. A 3-mil (0.076-mm.) tungsten wire is now introduced into the hole until it projects 8 mm. inside the flask. This ion-collecting wire is sealed in with picein wax.

The external surface of the unsilvered spherical segment is coated with a thin layer of ceresin wax. The remainder of the external surface of the flask is coated with colloidal graphite (Aquadag).

After the external portion of the collecting wire is cut to 3-mm. length (total length 1.3 cm.) the brass adapter is attached to the flask. This adapter is constructed so that when the entire apparatus is assembled the bottom of the flask is 2 mm. from the repelling post of the Lauritzen electroscope.

The adapter is fitted on to the electroscope, the collecting wire is brought near the repelling post by moving the quartz flask, and the flask is securely clamped to the adapter. Final adjustments are made by bending the collecting wire with a dissecting needle. A fine pencil brush is used for cementing the wire to the repelling post with colloidal graphite. During these operations the quartz fiber is kept charged in order to keep it out of the way. The four 9.5-mm. holes symmetrically drilled in the cylindrical sides of the adapter allow visual observation of the above manipulations, after which they are closed with rubber stoppers.

The electroscope chamber is made reasonably airtight by sealing the adapter to both the quartz flask and the electroscope mounting with beeswax and rosin. These nonconducting surfaces are then coated with colloidal graphite.

The detection apparatus is sealed to the rest of the vacuum system and the entire apparatus is ready for use.

A power pack of standard design, employing a 5R4 rectifier and three VR150 voltage regulator tubes, provides a constant 450-volt source required for operating the detection apparatus. A 115-volt pilot light (6-watt) illuminates the quartz fiber and eyepiece scale in spite of the interposition of the auxiliary ionization chamber.

The detection apparatus operates on the following principle:

Let the electroscope mounting, the brass adapter, and the Aquadag surfaces be grounded; let the silvered surface be maintained at a positive voltage; and let the unit, consisting of the gold-plated quartz fiber, the repelling post, and the ion-collecting wire be charged to a negative potential and then be isolated. Then the ion pairs (mainly CO_2^+ and e^-), produced by the inelastic collisions of beta-particles from C^{14} with gaseous carbon dioxide molecules inside the ionization chamber, are separated by the electric field resulting from the potential difference of the silvered surface and the collecting wire. The subsequent neutralization of the positive ions at the collecting wire makes the repelling post and quartz fiber less negative, and the elastic restoring force of the quartz moves the gold fiber somewhat nearer the repelling post. The rate of this motion is a measure of the amount of radioactivity present.

It is apparent that electrical leakage from the silvered and Aquadaged surfaces will also neutralize the charge on the repelling post. To prevent this the ionization chamber was made of quartz, in view of its high electrical resistance (*7*); as was to be expected (*7*), Pyrex proved to be unsatisfactory. Furthermore the ceresin wax minimized exterior surface conduction due to the absorption of water vapor on the quartz. No increase in "natural" background rate due to volume and surface electric conductivity of the quartz was detectable.

The absolute sensitivity of a Lauritzen electroscope depends upon the elastic force constant of the quartz fiber and the electrostatic capacity of the isolated unit. By connecting the collecting wire to the repelling post this capacity is increased by only about 0.1 mmf., since a wire of small diameter (0.076 mm.) and minimal length (1.3 cm.) is used. Depending somewhat upon the electroscope, the resulting reduction in sensitivity amounts to about 30%. It is suggested that electroscopes to be used for this purpose be ordered with a sensitivity of 5 to 6 divisions per minute per millicurie of radium at 1 meter.

Optimal operating conditions were ascertained by numerous preliminary tests.

With the brass adapter and Aquadaged surfaces grounded, the silvered surface inside the quartz ionization chamber is kept continuously at a potential of +240 volts. Except when measurements are being made, the collecting wire, repelling post, and fiber are held at a potential of −140 volts by means of a simple lock device attached to the electroscope charge button. These potentials are somewhat in excess of that necessary for maximum collection of ions. The continual maintenance of the above voltages is necessary because of the initial flow of polarization current or "soak in charge" into the quartz insulation; about 18 hours were required to attain complete equilibrium (*7*).

When not in use the quartz chamber is left filled with the last gas sample subjected to radioactive analysis. Under no circumstances is the chamber left evacuated more than 15 minutes. There is apparently at least a monomolecular layer of carbon dioxide that is strongly adsorbed by the quartz insulating the collecting wire from the silvered surface, and the orientation of this layer in the electrical field alters slightly the capacity of the measuring unit. This phenomenon is readily recognizable, since longer periods of evacuation will result in a discrete jump of about 3 small divisions of the electroscope fiber to a new position of equilibrium. Surface adsorption is indicated because this phenomenon does not take place with either dry air or hydrogen.

The negatively charged silvered fritted disk, through which the gas enters the ionization chamber, neutralizes the slow-moving polymeric molecular ions which are always present in carbon dioxide owing to cosmic ray bombardment. The presence of these ions in greater than nonequilibrium proportions results in an appreciably increased discharge rate of the electroscope fiber for all samples with activity the order of the background for a period of about 2 hours. The above ion trap enables immediate measurement of all samples. This ion trap proved to be as efficient as those of more usual design (*2*).

The ionization chamber is always filled with about 20 millimoles of carbon dioxide gas; since its volume is 219 cc., this is equivalent to about 2 atmospheres' pressure at room temperature. Under these conditions, the carbon beta-particles encounter sufficient inelastic collisions to produce 97% of the total ionization possible. Further increase in pressure to 3 atmospheres increases the efficiency to nearly 100%.

Sensitivity. Accurate radioactive analysis for C^{14} can be made by utilizing the information given above. Through the cooperation of W. W. Miller of the Massachusetts Institute of Technology, the number of counts per minute (±10%) were determined on 10-cm. mercury pressure of carbon dioxide containing C^{14} within a Geiger counter of 35 cc. detection volume. These data were then compared with that obtained with an identical sample inside the author's detection apparatus. For purposes of comparison, the relative sensitivity will be defined as the dimensionless ratio of measured radioactivity due to the sample to the measured activity due to background radiation; then, these data show that, even if it were possible to operate a 200-cc. Geiger counter

ble I. Radioactive Measurements as a Function of C^{14} Content of arbon Dioxide in the Ionization Chamber–Lauritzen Electroscope Detection Apparatus

Dilution experiments. The C^{14} concentration of the least active sample is ken as unity. Radioactive measurements are expressed as the ratio of the net activity to average background.)

Relative C^{14} Concentration, C_i	Radioactive Measurements Observed, R_i	Expected[a] R_i	Average Deviation, %
128	83.4		
	83.6		
	Av. 83.5	83.2	0.4
64	41.8		
	41.5		
	Av. 41.7	41.6	0.2
32	20.2		
	20.7		
	Av. 20.4	20.8	1.9
16	10.5		
	10.3		
	Av. 10.4	10.4	0.0
8	5.20		
	5.27		
	Av. 5.23	5.20	0.6
4	2.61		
	2.66		
	Av. 2.64	2.60	1.5
2	1.31		
	1.26		
	Av. 1.29	1.30	0.8
1	0.68		
	0.65		
	Av. 0.67	0.65	3.1

[a] $R_i = C_i \frac{\Sigma(R_i/C_i) \log C_i}{\Sigma \log C_i}$. This equation expresses the fact that the curacy of measurement increases somewhat with concentration of radioivity.

2 atmospheres carbon dioxide pressure, it would possess a relave sensitivity no greater than the authors' detection apparatus. hus an ionization chamber attached to a Lauritzen electroscope sufficiently sensitive for all investigations utilizing C^{14} as a acer, so long as there is adequate gas pressure to allow nearly aximum ionization within the chamber.

It is capable of detecting the presence of about 3×10^{-5} microrie of C^{14} in 20 millimoles of carbon dioxide (4 grams of barium rbonate). Surrounding the ionization chamber with a lead linder would decrease the minimum detectable quantities of ^{14} to about 2×10^{-5} microcurie. This method is of particular lvantage in biological work where 20 millimoles of carbon are rmally present in approximately 2 to 4 grams of tissue.

With the electroscope used, the discharge rate of the fiber when ie chamber is filled with 20 millimoles of nonradioactive carn dioxide (background) is numerically equal to about 4.3 small visions per hour. On the basis of 5 division determinations 0 minutes), the maximum variation in background was usually ss than 3% and seldom greater than 5%. This variation is mewhat less than that of the Lauritzen electroscope used preously (*4*), since the number of molecules inside the ionization amber, and thus the measured ionization resulting from each y, does not depend upon the room temperature and barometric essure; probably the number of alpha-particles emitted per uare centimeter of a silvered quartz flask is less than that of ass [~6 α's per sq. cm. per hour, (*1*)]; and unlike air (30 α's r hour per 100 cc.), the number of alpha-particles emitted from ire carbon dioxide is not measurable (*1*).

ANALYTICAL PROCEDURE

All organic compounds containing C^{14} are oxidized to carbon oxide by the standard combustion procedure. The issuing ses are dried with phosphorus pentoxide, and the carbon dioxide is condensed in a trap surrounded with liquid nitrogen. By means of a ground joint, this trap is then joined to an all-glass vacuum system similar in design to that reported in (*3*). The carbon dioxide is transferred to any desired part of the apparatus by means of vacuum distillation, employing liquid nitrogen as the condensing agent. The number of millimoles of gas produced is determined in a calibrated volume connected to a mercury monometer.

This carbon dioxide is transferred to a mixing bulb and the amount of nonradioactive (carrier) gas required to bring the total amount to 20 millimoles is added. The previous sample in the quartz ionization chamber is either transferred to a storage bulb or pumped away. The chamber is then completely evacuated for 5 minutes by means of a mercury diffusion pump. The carbon dioxide is condensed into tube *D* (Figure 1) and allowed to evaporate slowly through the ion trap into the ionization chamber. The electroscope charge button is now released and the discharge rate of the fiber is determined. Samples producing a sufficiently high discharge rate (greater than one small division per minute) are measured in triplicate; others are usually determined in duplicate. When the apparatus is in routine use, a standard sample, which is kept in a storage bulb attached to the vacuum system, is measured at the beginning and the end of the day; background is determined during the noon hour.

The accuracy of this method for the measurement of relative amounts of C^{14} was determined by progressive dilutions with inert carbon dioxide of a standard sample of carbon dioxide containing some C^{14}. Depending upon the activity present, each radioactive determination was made either in triplicate or duplicate. Two independent series of dilutions were measured. As in previous investigations (*4*), the data are presented in terms of the following equation:

$$R_i = \frac{(d/t)_s - (d/t)_b}{\overline{(d/t)_b}} \qquad (1)$$

where d is the distance traversed by the electroscope fiber in time t, s indicates the sample, b denotes background, and $\overline{(d/t)_b}$ is the average background rate of fiber discharge. The usual distance traversed by the fiber during measurements was 10 small divisions, although no samples were measured for periods longer than about 1 hour.

The experimental determinations for two independent dilution series are given in Table I. The concentration of the least active sample (approximately 5×10^{-5} microcurie of C^{14}) has been arbitrarily taken as unity.

The data indicate that radioactive analysis to 2% can be made on all carbon samples containing, per 20 millimoles of carbon, activity at least equal to the background.

ACKNOWLEDGMENT

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Analytical Procedure for Measurement of Radioactive Hydrogen (Tritium)

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A quantitative procedure for the measurement of radioactivity due to H^3 depends upon insertion of hydrogen gas in a quartz ionization chamber attached to a Lauritzen electroscope. Radioactive analysis of organic compounds containing H^3 is based on combustion to water and the quantitative conversion of this water to hydrogen. A procedure for obtaining tritium water samples of high specific activity is described.

TRITIUM (radioactive hydrogen) is in certain respects an ideal tracer element. It has a half-life of about 30 years (*7*) and samples of high millicurie strength are easily obtainable. The principal difficulty is in detection. Tritium (H^3) disintegrates into He^3 by the emission of a beta-particle of maximum energy (*6*) of about 0.015 Mev. This energy is so low that tritium must be detected in the gaseous phase.

Semiquantitative investigations using tritium as a tracer have been made principally by Ruben and co-workers (*8*), who analyzed for radioactivity by introducing hydrogen gas containing H^3 into a Geiger counter. Under the best conditions, radioactive analyses by this procedure are limited to about 10 to 15% accuracy because hydrogen is not an ideal counting gas (*1*); and because of the extreme importance of the nature of the cathode surface and traces of contaminants—e.g., mercury vapor—in the mechanism of Geiger counter discharge. It has been the authors' experience that the continual evacuation and refilling of counters are not practical as a routine procedure.

Black and Taylor (*3*) have obtained reproducible counting by introducing hydrogen at low pressure together with three contaminants into a counter. Unfortunately, their method is not adaptable to routine analysis.

Since certain planned investigations using tritium as a tracer necessitated about 2% accuracy, it was decided to employ a quartz ionization chamber attached to the Lauritzen electroscope as a means of analyzing the relative tritium content of hydrogen gas. This detection apparatus has been used successfully for the analysis of long-life carbon as carbon dioxide (*4*).

This paper evaluates this procedure as a general method for the radioactive analysis of compounds containing tritium as a tracer, and describes briefly the method of obtaining tritium samples of high specific activity.

PREPARATION OF TRITIUM SAMPLES OF HIGH SPECIFIC ACTIVITY

During the production of neutrons by the interposition of a water-cooled beryllium target in the deuteron cyclotron beam, two nuclear side reactions (*2*, *7*) take place which lead to the formation of radioactive hydrogen, H^3 (tritium, T). These reactions, the first of which gives the greater yield of H^3, are:

$$Be^9 + H^2 = H^3 + 2He^4(Be^8) \quad (1)$$

$$H^2 + H^2 = H^3 + H^1 \quad (2)$$

A significant fraction of this tritium remains trapped inside the beryllium target, probably as TD gas. The tritium is removed by placing the target in a quartz tube attached to a vacuum apparatus. The tube is evacuated, a predetermined quantity of "carrier" hydrogen is introduced, and the tube is then heated to red heat for about 15 minutes. The hydrogen is now oxidized to water by allowing the gas to come in contact with hot (300° C.) cupric oxide. The water is condensed out into a trap chilled with liquid nitrogen. At the completion of this reaction the degree of vacuum will be the same as before the introduction of the hydrogen, and thus a spark discharge serves as an indicator.

At least 99% of the tritium initially present in the target forms tritiated water by the above process. Both water and hydrogen are the most useful general starting materials for the synthesis of compounds containing tritium. In view of the ease of storage, water was chosen as the end product of the above process, especially since it is a simple matter to convert water quantitatively to hydrogen.

Three samples containing tritium have been prepared. In each case the amount of carrier hydrogen added was sufficient to form 2 millimoles (36 mg.) of water. Two of these preparations were made from beryllium targets obtained through the cooperation of J. G. Hamilton of the Radiation Laboratory, Berkeley, Calif. The authors are indebted to H. W. Fullbright of Washington University, St. Louis, for the beryllium scrapings of numerous targets employed in the preparation of the third sample. As data on the cyclotron bombardment times are not available, the millicurie strength of the water samples cannot be used to compute yield data.

DETECTION APPARATUS

All radioactive analyses are made on hydrogen gas containing tritium inside a quartz-ionization chamber attached to the Lauritzen electroscope. This apparatus has been used successfully for the detection of long-life carbon as carbon dioxide (*4*). The substitution of H^3 for C^{14} necessitated certain minor modifications.

Because of the low molecular weight of hydrogen, the ion trap is not necessary.

The chamber is filled with 10 millimoles of hydrogen, which is equivalent to a pressure of about 1 atmosphere. This quantity of gas is sufficient to ensure the production of all the ionization (mainly H_2^+ and e^-) which can result from the inelastic collisions of tritium beta-particles with hydrogen molecules.

The potentials of the silvered surface and the unit consisting of the collecting wire, repelling post, and quartz fiber are charged to

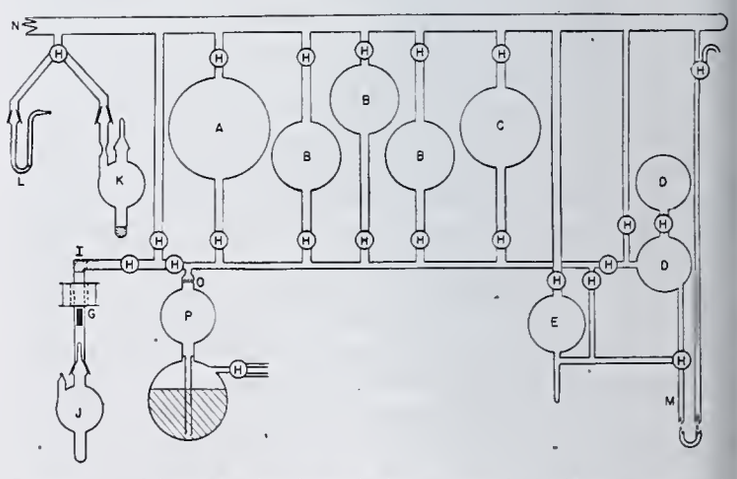

Figure 1. Vacuum Analytical Apparatus for Measurement of Tritium Content of Water

A. H_2 storage bulb (5 liters)
B. Storage bulbs (500 cc.)
C. Standard sample storage bulb (1 liter)
D. Mixing chamber
E. Ionization chamber attached to electroscope
G. Induction coil and hammer
H. Vacuum stopcock
I. Glass wool plug
J. Reaction flask in position to break seal
K. Reaction flask with Mg-Hg amalgam
L. U-tube with water sample containing tritium
M. Mercury manometer
N. To mercury vapor pump
O. Fritted disk medium porosity
P. Toeppler pump

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-125 and −125 volts, respectively. These potentials suffice or total collection of the ions.

Owing to the low ionization efficiency of hydrogen gas, the discharge rate of the electroscope fiber is exceedingly slow when the onization chamber contains 10 millimoles of nonradioactive ydrogen. Hence to facilitate the measurement of background nd "weak" samples, a 20× eyepiece containing a glass scale with .0254 mm. graduations is substituted for the standard 6× eyeiece and 0.1-mm. graduated scale.

For this particular detection apparatus, the background rates re numerically equal to about 1.2 and 5 small divisioos per hour, espectively, with the 0.1-mm. and 0.0254-mm. graduated scales.)n the basis of 1-hour determinations, the variations in this backround were identical with that obtained with carbon dioxide 4)—namely, a maximum variation that was usually less than %, and seldom greater than 5%. An intercomparison of this etection apparatus with a Geiger counter filled with hydrogen ontaining tritium showed that the relative sensitivity (activity/ ackground) of the ionization chamber is about 40% of that of a ounter of identical volume filled to the same pressure. Since ritium is readily obtainable with high specific activity, this reluction in relative sensitivity is trivial. The apparatus is capable f quantitative detection of about 10^{-4} microcurie of tritium in 0 millimoles of hydrogen.

In order to compare the dilution factors possible with tritium vith respect to those of the more commonly used hydrogen racer, deuterium, let us consider the tritium sample prepared rom targets obtained from Washington University at St. Louis. This sample, as prepared, contained about 1 millicurie of tritium n 2 millimoles of hydrogen; the dilution factor of this particular ample is at least 5×10^7. This dilution is about 100-fold greater han that possible with 100% deuterium. Since tritium containng considerably more than 1 millicurie total activity could be eadily prepared, this factor can still be increased significantly.

ANALYTICAL PROCEDURE

A general method for the determination of tritium activity is ased on the conversion of the hydrogen present in the sample to vater by the combustion method and the reaction of this water vith magnesium amalgam to form hydrogen. This hydrogen is hen introduced into the ionization chamber.

The quantitative formation of water by direct combustion of an organic compound in oxygen is standard. However, instead of absorbing the water vapor, it is condensed in a weighed U-tube chilled with a dry ice–ether mixture. The weight of water formed s then determined by reweighing the U-tube. The weight of water used for the production of hydrogen is about 220 ± 20 mg.; thus if the weight of the water formed by combustion is less than 200 mg., nonradioactive (carrier) water is added dropwise while the U-tube is on the balance; if this weight is greater than 240 mg., the water is well stirred and a rough aliquot is used.

Allen and Ruben (*1*) converted water to hydrogen by reaction vith magnesium at 620° C. The addition of mercury to the magnesium turnings lowers the temperature to 400° C. and a Pyrex vessel can be employed.

About 2 grams of magnesium turnings and 3 grams of mercury are introduced into a 250-cc. reaction flask (*K*, Figure 1) and are well mixed. The flask and U-tube containing the water are then attached to a 3-way stopcock by means of ground joints, as indicated in the upper left corner of Figure 1. The water is frozen with liquid nitrogen and this system is evacuated. All the water is vacuum-distilled into the reaction flask; the flask is then sealed off at the indicated constriction. Before placing the flask in an electric furnace, the contents are well shaken in order to disperse the magnesium amalgam throughout the inside surface. One hour at 400° C. suffices for complete production of hydrogen.

Storage bulb *A* contains nonradioactive hydrogen for the purpose of determining background. Storage bulb *C* contains a reference sample of tritium; during routine analysis this standard is measured daily and then toepplered back into *C*. The mixing chamber, *D*, enables the dilution of samples which are too active to measure accurately. The fritted disk, *O*, prevents spillage of the mercury in the Toeppler pump into the remainder of the vacuum system. The mercury manometer, *M*, measures the pressure of the hydrogen gas introduced into the ionization chamber, *E*. Since the volume of the ionization chamber and the manometer were calibrated, the number of millimoles introduced can be calculated.

Table I. Radioactive Measurements as a Function of Tritium Content in Benzene

(Dilution experiments. H^3 concentration in least active benzene sample is chosen as unity. Radioactivity measurements are expressed as ratio[a] of net activity to average background measured as hydrogen gas in ionization chamber–Lauritzen electroscope detection apparatus.)

Relative H^3 Concentration, C_i	Radioactive Measurements: Observed, R_i	Expected[b], R_i	Average Deviation, %
259	474		
	479		
	Av. 476	472	0.7
65.7	117.4		
	120.7		
	Av. 119	120	0.8
14.6	26.6		
	25.8		
	Av. 26.2	26.5	1.1
3.79	7.06		
	6.87		
	Av. 6.97	6.90	0.9
1.00	1.81		
	1.88		
	Av. 1.85	1.82	1.4

[a] This ratio includes factor $\frac{w_1 + w_2}{w_1}$ where w_1 is weight of water obtained from benzene combustion and w_2 is weight of nonactive water added to bring total weight above 200 mg. In each case this factor was about 2 and thus ratios of activities measured as hydrogen to background are ½ of the values given in table.

[b] $R_i = \frac{C_i \Sigma(R_i/C_i) \log C_i}{\Sigma \log C_i}$ expresses the fact that the accuracy of measurement increases somewhat with concentration of radioactivity.

The ionization chamber is always filled with 10 millimoles of hydrogen (about 70 cm.). In view of the unavoidable dead space during the toepplering of gas from the reaction flask, *J*, into the ionization chamber, *E*, at least 200 mg. (11 millimoles) of water must be used for conversion to hydrogen.

The relative amount of tritium present in the original sample analyzed is computed from the weight of water formed during the combustion process and any additional carrier water added, together with the discharge rates of the electroscope fiber when the ionization chamber is filled with the hydrogen generated from this water, the reference standard in bulb *C*, and nonradioactive hydrogen, respectively.

The threefold difference in mass between H^1 and H^3 may result in a considerable difference in reaction rates during any process involving either these atoms or the ions. In order to ensure completion of the chemical reactions in the above analytical procedure, radioactive analyses were made on radiobenzene progressively diluted with nonradioactive benzene. The radiobenzene was prepared by the nickel-catalyzed exchange between TH and the hydrogens of benzene; this method was used by Polanyi and co-workers for the partial deuteration of benzene (*5*).

In order to include in the final computations both the weight of the water produced by combustion and the weight of carrier water required to bring the total weight above 200 mg., about 160 mg. of benzene (about 6 millimoles of hydrogen) were analyzed in each case. These experiments also evaluate the quartz ionization chamber as an apparatus for the determination of relative concentrations of H^3.

The data are presented in terms of the following equation:

$$R_i = \frac{\frac{w_1 + w_2}{w_1}\left[(d/t)_s - (d/t)_b\right]}{(d/t)_b} \qquad (1)$$

where w_1 is weight of water resulting from the benzene combustion, w_2 is the weight of the carrier water added; d is the distance traversed by the electroscope fiber in time t, s indicates sample, and b denotes background. The usual distance traversed by the fiber during measurements was 10 small divisions, although no samples were measured for periods longer than about one hour; at least duplicate readings were made.

The radioactive analyses of duplicate benzene samples are given in Table I. The concentration of the least active tritiated benzene sample (approximately 3×10^{-4} microcurie of tritium) has been arbitrarily taken as unity.

The data in Table I indicate that no detectable isotope separation takes place in the analytical procedure given here. Furthermore, radioactive analysis to 2% can be made on organic compounds which contain enough tritium, per 10 millimoles of hydrogen gas, to give an activity at least equal to background.

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Determination of Hydroperoxides in Rubber and Synthetic Polymers

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The relative amounts of hydroperoxide in natural rubber or synthetic polymers can be determined by allowing the peroxide to react with ferrous iron in benzene-methanol solution, using o-phenanthroline as a color reagent for the excess ferrous iron. The method gives results reproducible to about 20% on a given rubber solution, but sampling difficulties may cause larger variations on solid samples. The procedure as written is sensitive to 10 or 20 p.p.m. of active oxygen, calculated on the solid polymer and it can be easily made sensitive to 1 p.p.m. by increasing the sample size and compensating for its color. Evidence is presented to show that the results with any ferrous iron procedure are high and have only relative significance.

FERROUS iron has been used as a reagent for the determination of peroxide by numerous investigators. Yule and Wilson (*12*) estimated peroxide in cracked gasoline by shaking with an acid solution of ferrous thiocyanate and back-titrating the resulting ferric salt with titanous chloride. The method was criticized by Young, Vogt, and Nieuwland (*11*), who used the color of the resulting ferric thiocyanate complex as a basis for a colorimetric determination of peroxide. In this procedure methanol was used as the solvent. Bolland *et al.* (*1*) modified the method to enable rubber samples to be analyzed by changing the solvent to a mixture of benzene and methanol (73% benzene by volume).

For determining peroxide in GR-S (butadiene-styrene copolymer) and other butadiene copolymers it would be desirable to employ a solvent rich in benzene, since these polymers tend towards insolubility upon oxidation. A solvent composed largely of benzene would also improve the sensitivity of the determination, since larger quantities of polymer could be dissolved in a given volume of solvent.

There are several disadvantages inherent in the use of thiocyanate as a color reagent for iron (*10*). The intensity of the color depends upon the concentration of thiocyanate and upon the acid concentration, and varies with the presence of various ions which form complexes with iron. In practice, a large excess of thiocyanate is used to lower the effects of acid and interfering ions and to give a closer approximation to Beer's law. The red color of the ferric thiocyanate complex is somewhat unstable, requiring colorimeter readings to be made without undue delay. The ferrous thiocyanate reagent of Bolland *et al.* (*1*) and Robey and Wiese (*7*) is so unstable that it must be freshly prepared every few hours and stored in the dark.

The advantages of *o*-phenanthroline as a color reagent for iron have been recognized by many investigators (*3*, *8*, *9*, *10*). Its complex with ferrous iron is very stable, its solutions showing no change in color for many months. Beer's law is closely obeyed. The intensity is not so strongly affected by the concentration of excess reagent or of acid, as in the case of the thiocyanate complex. Since large concentrations of inorganic salts are not needed it should be possible to use a solvent consisting largely of benzene, with only a small amount of methanol. Solutions of ferrous salts in methanol under nitrogen and *o*-phenanthroline in benzene are stable and can be stored indefinitely without noticeable change.

The effects of complexing agents on the oxidation potentials of the ferrous-ferric ion system are of interest. Thiocyanate lowers the oxidation potential, making the oxidation of the ferrous ion proceed more readily. This shift is advantageous in making the reagent very reactive toward polymer peroxides but also accounts for its extreme sensitivity to air oxidation. Phenanthroline has just the opposite effect (*4*); the ferrous phenanthroline complex is not appreciably oxidized by polymer peroxides, and it is entirely stable with respect to air oxidation. To increase the reactivity with polymer peroxides, uncomplexed ferrous iron was first tried as a reagent. As will be seen below satisfactory results were obtained under the proper conditions provided that no antioxidant was present in the polymer. In the presence of antioxidant, incomplete reduction of rubber peroxide was observed. It was found that the addition of phosphoric acid (which lowers the ferrous-ferric oxidation potential) made the reaction proceed satisfactorily even in the presence of antioxidant. Nitric acid was added to prevent a color due to solvolysis of ferric iron.

Farmer and Sutton (*2*) observed that natural rubber forms only peroxides of the hydroperoxide type. In the present investigation, numerous synthetic polymers, including polyisoprene, polybutadiene, and copolymers of isoprene or butadiene with styrene, acrylonitrile, and other vinyl-type monomers, were found to form peroxides which reacted at the same rate as natural rubber peroxides with the text reagent. Since disubstituted organic peroxides failed to react (*2*), this observation is evidence that all the polymers studied form peroxides of the hydroperoxide type.

PROCEDURE

This method is intended for the determination of relative amounts of hydroperoxides in natural rubber, and various synthetic polymers and copolymers. It is applicable only to hydroperoxides, and except for this limitation it can be used generally for compounds of low molecular weight as well as high polymers.

Preparation of Solutions. *Ferrous Iron Solution.* Remove the dissolved oxygen from 1 liter of methanol by bubbling nitrogen through it for 30 minutes. Dissolve 19.605 grams of ferrous ammonium sulfate hexahydrate, $FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$

n the methanol containing 13.9 ml. of concentrated sulfuric cid to make the solution about 0.25 *M* in sulfuric acid. If stored nder an atmosphere of nitrogen, this solution is stable for a long ime. For use over a period of a few days, 10-ml. portions can be llowed to stand in air.

Each day a 0.002 *N* working solution is prepared by diluting ml. of the 0.05 *N* solution to 50 ml. with methanol but without dding acid.

Nitric Acid–Methanol Solution. Add 1 ml. of concentrated itric acid to 20 ml. of methanol slowly and with cooling. One illiliter of this solution provides 0.05 ml. of concentrated nitric cid.

Phosphoric Acid–Methanol Solution. Prepare a 1 *M* stock solution by adding 6.75 ml. of 85% phosphoric acid (14.8 *M*) to ufficient methanol to prepare 100 ml. of solution. Dilute the *M* solution to 0.04 *M* for use as a working solution; 0.5 ml. of .04 *M* solution provides 0.02 millimole of phosphoric acid.

o-Phenanthroline Solution. Dissolve 1 gram of *o*-phenanthroine monohydrate in 1000 ml. of thiophene-free benzene.

Analysis of Unknown Samples. Pipet 1 ml. of rubber solution (containing about 1 gram of polymer per 100 ml. of benzene) nto a 50-ml. volumetric flask. Add about 25 ml. of thiophene-free enzene, washing any polymer solution from the sides of the flask. dd 1 ml. of nitric acid–methanol solution and 0.5 ml. of 0.04 *M* hosphoric acid (methanol), shaking well after each addition. ipet 1 ml. of 0.002 *M* ferrous iron into the flask, shake well, and llow the mixture to stand at least 15 minutes. Add 3 to 5 ml. of -phenanthroline solution and make up to 50 ml. with thiophene-ree benzene. Read the transmission value at 508 mμ with a spectrophotometer or use a photoelectric colorimeter provided with a ilter showing maximum transmission at 500 or 510 mμ (preferably not 520 mμ), comparing the transmission with that of enzene.

If the transmission value lies between 50 and 70%, calculate he active oxygen content as shown below. Otherwise, estimate he adjustment necessary in sample size required, and repeat the etermination. For samples of entirely unknown peroxide content, it is advisable to vary the sample size, using 0.1, 1, and 10 nl. of polymer solution in the preliminary estimation. If a 10-nl. sample is used, or if the sample is highly colored, a diluted enzene solution of polymer should be used instead of benzene for he comparison liquid in the optical measurement.

Construction of Calibration Curve. Using a graduated -ml. pipet, add the following amounts of 0.002 *N* ferrous iron olution to a series of 50-ml. volumetric flasks: 0.2, 0.4, 0.6, 0.8, nd 1.0 ml. Add about 25 ml. of benzene to each flask, washing he walls carefully. Complex the ferrous iron with 3 to 5 ml. of -phenanthroline solution and make up to 50 ml. with benzene. ake colorimeter or spectrophotometer readings relative to benene.

Compute the optical density of each solution from the equation $D = \log I_0/I$, and construct a graph of optical density against concentration of iron (expressed as equivalents of ferrous iron per 50 ml.). A straight line, corresponding to Beer's law, should be obtained.

Table I. Effect of Sulfuric Acid on Ferric Complex

0.002 *N* Iron Complexed	Concentrated H_2SO_4	Time of Standing		Transmission	
Ml.	*Ml.*	t_1, *min.*	t_2, *min.*	I_1, %	I_2, %
1.0	0.00	95	1200	87	85
1.0	0.02	95	1200	98	97
1.0	0.05	95	1200	99.5	98.5
1.0	0.08	95	1200	99.5	99
1.0	0.0	90	360	81	78
1.0	0.1	90	360	100	99
1.0	0.2	90	360	100	100
1.0	0.4	90	360	100	100
3.0	0.0	90	1200	80	80
3.0	0.1	90	1200	99	78
3.0	0.2	90	1200	98.5	98
3.0	0.4	90	1200	99	99

Table II. Effect of Sulfuric Acid on Ferrous Complex

0.002 *N* Iron Complexed	Concentrated H_2SO_4	Time of Standing	Transmission
Ml.	*Ml.*	*Min.*	%
1.0	0.00	45	34
1.0	0.02	45	62
1.0	0.05	45	68.5
1.0	0.08	45	69
1.0	0.0	45	34
1.0	0.1	45	62
1.0	0.2	45	65
1.0	0.4	45	69

Table III. Effect of Nitric Acid

0.002 *N* Iron Complexed	Concentrated HNO_3	Time of Standing		Transmission	
Ml.	*Ml.*	t_1, *min.*	t_2, *min.*	I_1, %	I_2, %
		On Ferric Complex			
1.0	0.00	15	60	94	94
1.0	0.02	15	60	95	95
1.0	0.05	15	60	95	95
1.0	0.08	15	60	95	94.5
1.0	0.	960	..	76.5	..
1.0	0.2	960	..	96	..
1.0	0.5	960	..	96	..
1.0	1.0	960	..	98	..
		On Ferrous Complex			
1.0	0.00	15	60	34	34
1.0	0.02	15	60	34	34.5
1.0	0.05	15	60	34	34.5
1.0	0.08	15	60	34	34.5
1.0	0.0	960	..	34	..
1.0	0.1	960	..	36	..
1.0	0.3	960	..	83	..
1.0	0.5	960	..	95	..

Calculations. From the calibration curve the amount of iron remaining unoxidized can be read directly. By difference the amount of iron oxidized is determined. Each equivalent of iron oxidized corresponds to one equivalent or 8 grams of active oxygen. The active oxygen content is calculated as parts per million parts of solid polymer. If a polymer is only partly soluble, the concentration of the soluble polymer is determined by evaporation of an aliquot portion of the solution, and the calculation is based on the soluble part only.

$$\frac{(A - B) \times 8 \times 10^6}{C \times V} = \text{micrograms of active oxygen per gram of polymer} = \text{p.p.m. active oxygen}$$

where A = equivalents of ferrous iron taken
B = equivalents of ferrous iron remaining
C = concentration of polymer grams per ml. of benzene
V = ml. of polymer solution used

DEVELOPMENT OF METHOD

Spectrophotometric Curves of Standard Iron Solutions. A 0.05 *N* stock solution of ferrous iron was prepared by dissolving ferrous ammonium sulfate, $FeSO_4(NH_4)_2SO_4.6H_2O$, in air-free methanol, with sufficient concentrated sulfuric acid added to make the solution 0.25 *M* in acid. The solution was stored under nitrogen and by occasional titration with ceric sulfate was found to be stable indefinitely. A 0.002 *N* working solution was freshly prepared each day by diluting 2 ml. of the stock solution to 50 ml. with methanol.

A 0.1% solution of *o*-phenanthroline in benzene was used as the indicator solution. Various amounts of iron from 2.5×10^{-4} to 2.5×10^{-3} milliequivalent were treated with 6 ml. of indicator solution, and diluted to 25 ml. with benzene, and the absorption curves were determined with a Cenco-Sheard spectrophotelometer. In agreement with Fortune and Mellon (*3*) for aqueous solutions, the maximum absorption was found at 508 mμ.

Calculation of the optical density at the wave length of maximum absorption and plotting against concentration showed that Beer's law was being closely followed over the concentration range studied.

Effect of Acids on Ferrous and Ferric Complexes. Preliminary experiments showed that while the concentration of excess indicator could be varied over wide limits without affecting the absorption at 508 mμ, the acidity of the solution is of considerable importance. If the acidity is too low, the ferric ion shows appreciable absorption, apparently because of partial solvolysis of the *o*-phenanthroline complex. Upon complete oxidation of a solution of ferrous iron, the usual yellow color of ferric iron appeared. When *o*-phenanthroline was added, a deeper yellow color

Table IV. Effect of Time, Temperature, and Acid on the Reaction of Rubber Peroxides and Ferrous Iron

Concentrated HNO_3	Temperature	Time of Reaction	Time after Complexing		Transmission	
Ml.		*Min.*	t_1, *min.*	t_2, *min.*	I_1, %	I_2, %
0.1	Cold	5	30	..	64	..
0.1	Cold	10	30	..	67.5	..
0.1	Cold	15	30	960	69	64
0.1	Cold	20	30	..	69.5	..
0.1	Cold	30	55	180	69.5	68
...	Cold	30	40	...	64	..
0.1	Hot	15	30	960	66	62
...	Hot	15	30	960	67	60
0.1	Hot	30	30	960	70	66
...	Hot	30	30	960	70	64
0.1	Hot	60	30	120	70	69.5

Table V. Effect of Concentration of Excess Ferrous Iron

Polymer Solution	0.002 N Ferrous Iron	Time after Complexing		Transmission		Active[a] Oxygen
		t_1,	t_2,	I_1,	i_2,	
Ml.	*Ml.*	*min.*	*min.*	%	%	*P.p.m.*
0.2	1.0	10	180	43.5	43	2230
0.4	1.0	10	180	51	50	1730
0.6	1.0	10	180	59	57	1550
0.8	1.0	10	..	67	..	1470
1.0	1.0	10	..	70	..	1220
1.2	1.0	10	..	77	..	1150
1.4	1.0	10	..	83	..	1080
0.4	0.4	60	..	86	..	1380
0.4	0.8	60	..	61	..	1540
0.4	1.0	60	..	51	..	1730
0.4	1.2	60	..	40	..	1610
1.0	0.8	30	..	82.8	..	1120
1.0	1.0	30	..	70.0	..	1220
1.0	1.2	30	..	62.5	..	1390
1.0	1.4	30	..	47.0	..	1280

[a] Calculated as parts of active oxygen (equivalent weight = 8) per million parts of dry polymer.

appeared instead of the usual blue color obtained in aqueous solutions. With a larger excess of acid, absorption at 508 mμ by the ferric complex could be eliminated, but the ferrous *o*-phenanthroline complex then became unstable.

A series of experiments was performed in which conditions were sought that would give the maximum transmission for ferric iron and the minimum for ferrous iron at 508 mμ. In each experiment a known amount of standard ferrous ammonium sulfate in methanol or standard ferric chloride, prepared by dissolving sublimed ferric chloride in methanol, was used. The iron solution, in a 50-ml. volumetric flask, was treated with 25 ml. of benzene. Five milliliters of 0.1% benzene solution of *o*-phenanthroline were added. After addition of the acid being investigated and just sufficient methanol (1 to 2 ml.) to keep the acid in solution, the volume was made up to 50 ml. with benzene. The spectrophotometric transmission value was determined at 508 mμ at different time intervals after complexing, using benzene as a comparison liquid.

The results for sulfuric acid are shown in Tables I and II and for nitric acid in Table III.

Similar data for trichloroacetic acid showed that the ferrous complex was unaffected but that the absorption by the ferric complex could not be removed. Hydrochloric acid prevented absorption by the ferric complex but decomposed the ferrous complex.

Nitric acid is the only acid tried that will remove the absorption by the ferric complex at 508 mμ and yet not affect the ferrous complex. Not more than 0.1 ml. of concentrated acid can be used per 50 ml. of solution. Using this amount of acid, the absorption by the ferrous complex is not affected, and only 5% absorption is found for the ferric complex.

Effect of Time, Temperature, and Acid on Reaction between Polymer Peroxide and Ferrous Iron. A polymer of relatively high peroxide content was desired.

A laboratory GR-S sample was heated in air at 70° for 2 hours. The polymer was allowed to stand with 100 ml. of benzene for each gram of polymer: the gel was filtered off with a 100-mesh stainless steel screen. The resulting solution contained 0.0088 gram of soluble polymer per ml. Preliminary experiments showe that 1 ml. of this polymer solution gave a convenient extinctio value when allowed to react with 1 ml. of 0.002 N ferrous iron.

Experiments were run as described in the previous section, usin 1 ml. of polymer solution, 1 ml. of 0.002 N ferrous iron, and 0. ml. of nitric acid, except as noted. The solutions containin ferrous iron, polymer, and acid were allowed to stand variou lengths of time at room temperature before the *o*-phenanthrolin was added. No oxidation of iron occurred in the presence of th indicator. In certain experiments the mixture was heated t boiling and kept at the boiling point for the desired time. I these instances the acid was not added until after cooling an complexing, as the acid would oxidize the ferrous iron in hot solu tions.

The data in Table IV indicate that the reaction between poly mer peroxide and ferrous iron is complete in 15 minutes at roo temperature if acid is present. No increase in transmission wa obtained by heating up to 60 minutes. The final solutions wer stable with respect to transmission values for periods of 1 to hours, with only slight effects over longer periods of time. In a cases a more stable color was obtained in the presence of acid.

Effect of Concentration of Excess Ferrous Iron. T determine whether an excess of ferrous iron is necessary for com plete reduction of the rubber peroxides, the same polymer solu tion and procedure were used as in the previous section, allowin reaction times of 15 and 30 minutes at room temperature. Th results are given in Table V.

From Table V it appears that with an increasing excess o ferrous iron a higher apparent peroxide content is in general ob tained. As is shown below, this trend is caused by a variabl amount of air oxidation of the excess ferrous iron. By limitin the observed transmission range to 50 to 70%, a maximum devi ation of around ±20% in apparent peroxide content is allowe according to Table V. Considering the difficulty of samplin solid polymers and the relative nature of the method, this var ation is not serious for following trends in polymer peroxides.

Effect of Antioxidant. To determine the effect of anti oxidant, 2% of phenyl-β-naphthylamine (based on the dry poly mer) was added to the polymer solution already studied. Wit antioxidant present, hardly any oxidation of the ferrous iro occurred either at room temperature or upon heating.

Effect of Phosphoric Acid in Preventing Interferenc by Antioxidant. Experiments designed to increase the ease o oxidation of ferrous iron by lowering the ferrous-ferric ion ox dation potential were carried out. Fluoride and phosphoric aci were used as complexing agents. After preliminary experiment phosphoric acid was adopted to avoid the etching effect o fluorides. The optimum concentrations of phosphric and nitri acids were found by a study of the spectrophotometric absorp tion at 508 mμ of the ferrous and ferric *o*-phenanthroline com plexes in the absence of peroxides. The same procedure was use as for ferrous and ferric complexes above, with the addition o phosphoric acid in the form of a methanol solution. The result appear in Table VI. In each experiment 1.00 ml. of 0.002 N fe rous or ferric iron was treated with various amounts of pho phoric and nitric acids, *o*-phenanthroline was added, and th volume brought to 50 ml.

Even small amounts of phosphoric acid exert an adverse effe on the ferrous complex if no nitric acid is present, presumably b pulling the ferrous iron away from the *o*-phenanthroline comple A small amount of nitric acid, by suppressing the ionization of th phosphoric acid, prevents the interference. Phosphoric acid ha a desirable effect on the ferric iron in decreasing the absorption b forming the colorless ferric phosphate complex. From these re sults it was decided that 0.02 millimole of phosphoric acid an 0.05 ml. of concentrated nitric acid per 50 ml. are the optimu amounts.

To determine whether phosphoric acid prevents the interfe ence by phenyl-β-naphthylamine, the polymer solution contain ing antioxidant was tried with a reaction time of 20 minutes befor adding the indicator. The results are shown in Table VII.

Table VI. Effect of Phosphoric Acid

Concentrated HNO_3	H_3PO_4	Time of Standing		Transmission	
Ml.	*Millimoles*	t_1, *min.*	t_2, *min.*	T_1, %	T_2, %
		On Ferrous Complex			
0.0	0.00	30	...	34	..
0.0	0.008	30	...	40.3	..
0.0	0.02	30	...	43.3	..
0.0	0.04	30	...	44.3	..
0.05	0.00	20	180	34.0	33.0
0.05	0.008	20	180	33.9	35.0
0.05	0.02	20	180	34.0	35.9
0.05	0.04	20	180	34.5	36.5
0.1	0.00	25	180	35	36
0.1	0.008	25	180	36	39
0.1	0.02	25	180	36	39
0.1	0.04	25	180	36	39
		On Ferric Complex			
0.05	0.00	35	...	95	..
0.05	0.008	35	120	97	97
0.05	0.02	35	120	97	97
0.05	0.04	35	120	98	98

Table VII. Oxidation of Ferrous Iron in Presence of Antioxidant

Polymer Solution	0.002 *M* Ferrous Iron	Time after Complexing	Transmission	Active Oxygen
Ml.	*Ml.*	*Min.*	%	*P.p.m.*
0.2	1.0	15	44	2230
0.4	1.0	15	51.5	1770
0.6	1.0	15	59.2	1560
0.8	1.0	15	67	1420

omparison of the results with those obtained with the same olymer solution in the absence of antioxidant (Table V, first our experiments) shows that very similar data were found in the wo cases. The modified method using both nitric and phosphoric acids was also tried on the polymer solution without ntioxidant, with very similar results.

The commonly used antioxidants BLE (a complex condensation product of acetone and diphenylamine) and Stalite (an lkylated secondary arylamine) were likewise shown not to interere in the peroxide determination.

ABSOLUTE ACCURACY

To check the absolute accuracy of the proposed method, an nalysis of a standard peroxide was desired. Benzoyl peroxide nd furoyl peroxide, which is much less stable thermally, failed o react noticeably with the reagent even after long standing. n the other hand, *tert*-butyl hydroperoxide reacted as readily as olymer peroxides.

Since Farmer and Sutton (*2*) have shown natural rubber peroxides to be of the hydroperoxide type, it was of interest to compare the results obtained on rubber peroxides by the proposed ethod and by the thiocyanate method of Robey and Wiese (*7*), hich responds to all peroxides. The same rubber or GR-S solution showed about 20% more peroxide (relative) by the *o*-phenanthroline method than by the thiocyanate method.

Analysis of solutions of *tert*-butyl hydroperoxides standardized y the iodide method of Liebhafsky and Sharkey (*6*), and diluted or colorimetric analysis, showed that both colorimetric procedures gave high results, increasing with decreasing amounts of eroxide in the reaction mixture (compare Table V). However, areful exclusion of oxygen by bubbling the reagents before and uring the reaction led to lower results (Table VIII). With use of he thiocyanate method, the rate of reaction between peroxide and ron was found to decrease markedly as the last traces of oxygen ere removed. Thus a fading of the thiocyanate color led to low esults in air-free solutions. These observations suggest that eroxide catalyzes the air oxidation of iron (induced reaction) and onversely that oxygen catalyzes the peroxide iron reaction. Thus oth colorimetric procedures are nonstoichiometric in the presnce of air and very slow in its absence. For best results, both

Table VIII. Effect of Air on Colorimetric Peroxide Determination

0.002 *N* Peroxide	0.002 *N* Iron	Iron Used	Recovery	Conditions
Ml.	*Ml.*	*Ml.*	%	
		o-Phenanthroline Method		
0.50	1.20	1.14	228	Air saturated
0.50	1.00	0.73	140	N_2 through benzene only
0.50	1.00	0.65	130	N_2 through all reagents before reaction
0.50	1.00	0.59	118	N_2 through all reagents and during reaction
		Thiocyanate Method		
0.20	15	0.38	190	Air saturated
0.30	15	0.56	187	Air saturated
0.50	15	0.75	150	Air saturated
0.80	15	0.77	96	Air saturated
0.30	15	0.55	183	N_2 before reaction
0.50	15	0.72	144	N_2 before reaction
0.80	15	0.74	93	N_2 before reaction
0.30	15	0.18	60	N_2 before and during reaction
0.50	15	0.12	24	N_2 before and during reaction

should be applied only over a narrow range of peroxide content in the reaction mixture.

If the recommended transmission range of 50 to 70% is used in the proposed procedure (corresponding to a twofold range of peroxide concentration), comparative analyses can readily be made without standardization. For results of absolute significance, standardization by means of *tert*-butyl hydroperoxide is recommended. For larger amounts of peroxides, the iodide method gives stoichiometric results, but titration methods involving ferrous iron again were found to give high results in the presence of air. This observation has also been made by Kolthoff and co-workers (*5*) in the analysis of soap peroxides.

SENSITIVITY

The procedure as given is sensitive to 10 or 20 p.p.m. active oxygen, calculated on the solid polymer. No particular effort has been made to gain the utmost sensitivity, but much larger sample sizes could be used. The limiting factor is the color of the polymer solution itself, which could be compensated by using a solution of the polymer rather than benzene as the comparison liquid. By this means, less than 1 p.p.m. of active oxygen could be detected, considering the fact that the method gives an abnormal response to very small traces of hydroperoxide.

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Separation of Calcium from Magnesium

By Oxalate Method in Samples of High Magnesium-Calcium Ratio

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By careful control of the relative concentrations of magnesium and oxalate ion, it is possible to achieve acceptable separations of calcium from magnesium by direct precipitation of calcium oxalate in samples containing magnesium-calcium ratios of 20 to 1 or greater. Results which can be achieved with a single precipitation are limited by the solubility of calcium oxalate which increases rapidly with increasing oxalate concentration in hot solutions.

IN THE analysis of magnesites and magnesium chloride brine concentrates, it has long been recognized that the separation of calcium as calcium oxalate becomes increasingly uncertain as the magnesium content of the sample is increased. This uncertainty is caused by two properties of magnesium oxalate: its complex-forming nature which effectively ties up oxalate ion and makes the precipitation of calcium oxalate slow or incomplete, and its low solubility which causes excessive adsorption and especially postprecipitation of magnesium oxalate.

As a result of a study of the solubility of magnesium oxalate in ammonium oxalate solutions, Bobtelsky and Malkowa-Janowski (*2*) pointed out that both these effects could be ameliorated by adding to the solution a much larger quantity of oxalate ion than normally employed, the additional concentration of oxalate having the effect of forming a soluble complex with magnesium and furnishing sufficient oxalate in excess for the precipitation of the calcium. Additional information is herein presented dealing with the solubility of calcium oxalate, the quantity of oxalate required to prevent precipitation of magnesium oxalate, the best mode of precipitation of calcium oxalate, the amount of error to be expected from coprecipitation of magnesium, and maximum permissible magnesium-calcium ratio.

REAGENTS

MAGNESIUM CHLORIDE SOLUTION. A solution of magnesium chloride free of calcium was prepared by dissolving pure magnesium metal in hydrochloric acid and diluting so that it contained 16.0% magnesium chloride (4.09% magnesium). A spectrographic analysis showed that this solution contained less than 0.001% calcium and no detectable amount of aluminum, copper, iron, manganese, silicon, or nickel. The solution was stored in a Pyrex, glass-stoppered bottle.

STANDARD CALCIUM SOLUTION. The solutions were made up as needed to contain about 0.8 mg. of calcium per millimeter by dissolving a weighed quantity of Baker's Iceland spar in a slight excess of distilled hydrochloric acid and diluting to known volume. The solutions were standardized according to the procedure shown below, although a good agreement was always obtained between the calcium content as determined and that obtained by assuming the Iceland spar to be a primary standard.

HYDROCHLORIC ACID. Reagent grade hydrochloric acid was diluted with an equal volume of water and distilled from an all-Pyrex still. The resulting solution was approximately 6 *N* in hydrochloric acid.

AMMONIA. Ammonia solutions were prepared 1 liter at a time by passing ammonia gas from a cylinder through a water scrubber, then into cold water in a Vycor flask. The concentrated solution (6 to 9 *N*) so obtained was stored in the Vycor flask, or in some cases in hard rubber bottles.

AMMONIUM OXALATE: For most of the work, ammonium oxalate was purified by a single crystallization from hot water in order to remove insoluble impurities.

In some cases where crystallization did not appear necessary, analytical reagent grade ammonium oxalate monohydrate was used directly. Determinations of the solubility of calcium oxalate were made with ammonium oxalate prepared by crystallizing reagent grade oxalic acid which was then neutralized with ammonia gas.

NITRIC ACID. Analytical reagent grade acid was distilled from an all-Pyrex still and stored in a Pyrex bottle.

PROCEDURE

STANDARDIZATION OF CALCIUM SOLUTIONS. The required amount (25.00 or 50.00 ml.) of calcium solution was pipetted into a 400-ml. beaker and 5 ml. of 6 *N* hydrochloric acid were added. The solution was diluted to 75 ml., 25 ml. of 5% ammonium oxalate solution were added, and the solution was heated to about 80° C. The calcium oxalate was then precipitated by the dropwise addition of 6 *N* ammonia until the solution became basic to methyl red indicator and about 3 drops in excess. After digestion at 80° C. for about 15 minutes the solution was allowed to cool for an hour, and was then filtered through a tared fritted Pyrex filter (fine porosity) and washed 5 times with cold 0.2% ammonium oxalate solution. The precipitate was dried for a half hour at 180° C., and was ignited for one hour at 475° to 500° C. in a muffle furnace according to the procedure of Willard and Boldyreff (*9*). The resulting calcium carbonate was allowed to cool at least 4 hours in a desiccator containing anhydrous calcium chloride and was then weighed.

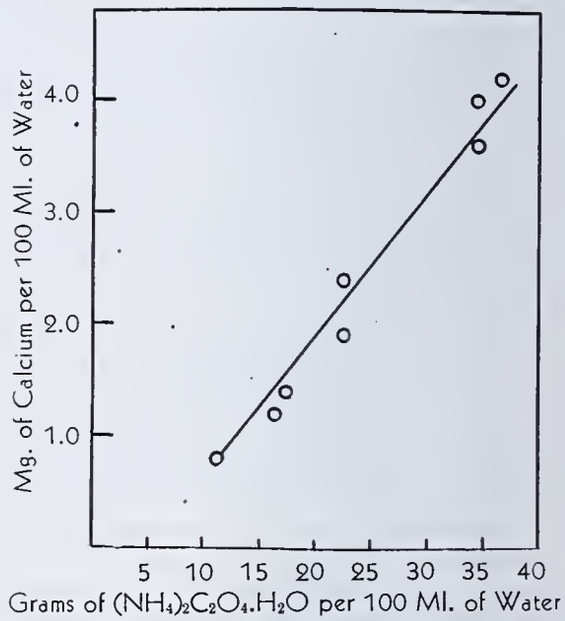

Figure 1. Solubility of Calcium Oxalate in Ammonium Oxalate Solutions at 95° C.

Table I. Solubility of Calcium Oxalate in Solutions of Ammonium Oxalate and Other Salts

Salt Present per 100 Ml. of H_2O at 80–90° C.	Calcium Carbonate Taken	Calcium Carbonate Found	Ca Dissolved
Grams	*Mg.*	*Mg.*	*Mg.*
5.0 $(NH_4)_2C_2O_4.H_2O$	49.5	49.2	0.14[a] ± 0.02
10.0 $(NH_4)_2C_2O_4.H_2O$	49.5	48.6	0.35[a] ± 0.05
15.0 $(NH_4)_2C_2O_4.H_2O$	49.4	47.9	0.60[b] ± 0.12
20.0 $(NH_4)_2C_2O_4.H_2O$	57.0	53.6	1.4[c] ± 0.2
25.0 $(NH_4)_2C_2O_4.H_2O$	49.5	44.4	2.0[a] ± 0.2
18.0 $H_2C_2O_4.2H_2O$[d]	56.5	53.2	1.4[e]
26.0 $K_2C_2O_4.H_2O$	68.4	63.2	2.0[e]
20.0 NH_4Cl	56.5	56.4	<0.1[e]
20.0 NaCl	56.5	56.8	<0.1[e]
20.0 KCl	56.5	56.8	<0.1[e]

[a] Average of four determinations.
[b] Average of three determinations.
[c] Average of five determinations.
[d] Neutralized with NH_4OH.
[e] Average of two determinations.

PRECIPITATION FROM MAGNESIUM CHLORIDE SOLUTIONS. veral modes of precipitation were tried as shown below, the llowing being adopted finally as the recommended procedure.

The neutral magnesium chloride solution containing the oper amount of calcium and about 1 gram of ammonium chlo- de is diluted to 100 ml. in a 400-ml. beaker and heated to 80° C. he proper quantity of solid ammonium oxalate monohydrate om Table III) is then added all at once and the mixture irred until solution is complete. The precipitate is digested for 5 to 1 hour at 80° C., and filtered hot through a tared Pyrex ter. The determination is completed as for standardization of lcium solution.

In many cases a second precipitation was made by dissolving e ignited calcium carbonate in dilute hydrochloric acid, filtering e acid solution through the Pyrex filter from which the calcium rbonate was dissolved, and then continuing by the procedure own above under standardization of the calcium solutions.

RESULTS

SOLUBILITY OF CALCIUM OXALATE. The fact that the solu- lity of calcium oxalate increases with oxalate-ion concentra- on became evident early in the work, and in order to obtain ta showing the magnitude of this effect, a number of deter- inations were made by adding various quantities of solid monium oxalate monohydrate to the hot solution containing lcium oxalate monohydrate precipitated by the procedure scribed above for standardization of the standard calcium lutions. After addition of the solid ammonium oxalate the lutions were digested hot for 1 hour, filtered hot, and washed th cold 0.2% ammonium oxalate. Results are shown in able I, together with similar determinations made with a few her salts.

In order to provide further corroboration of the general magni- de of this solubility, a number of determinations were made by alyzing for calcium in the filtrate of the ammonium oxalate lutions. For this purpose an all-Pyrex apparatus was em- ployed containing a sealed-in fritted filter, so that the filtration could be made while the apparatus was immersed in a constant-temperature bath at 95° C. Results by this method (Figure 1) provide an approximate check on those values shown in Table I.

It is evident from these solubility figures that if hot filtration is employed, the solubility error is appreciable and increases rapidly with increasing oxalate concentration.

QUANTITY OF AMMONIUM OXALATE REQUIRED. The solubility at 100° C. of magnesium oxalate dihydrate in ammonium oxalate solutions of concentration up to 20 grams of ammonium oxalate per 100 grams of solution is shown in Figure 2 according to data presented by Bobtelsky and Malkowa-Janowski (*2*).

The practical significance of these solubility figures was checked approximately as shown in Table II by a procedure which consisted of adding various quantities of solid ammonium oxalate monohydrate to 100 ml. of hot solution containing known quantities of magnesium and calcium. These solutions were digested at 80° to 90° C. for 0.5 to 1 hour and filtered hot. The quantity of precipitated magnesium oxalate drops sharply when sufficient ammonium oxalate is present to equal the solubility requirement shown in Figure 2. In actual practice a further excess of oxalate appears desirable in reducing coprecipitation of magnesium; consequently amounts of ammonium oxalate are recommended as shown in Table III, together with the solubility corrections necessary.

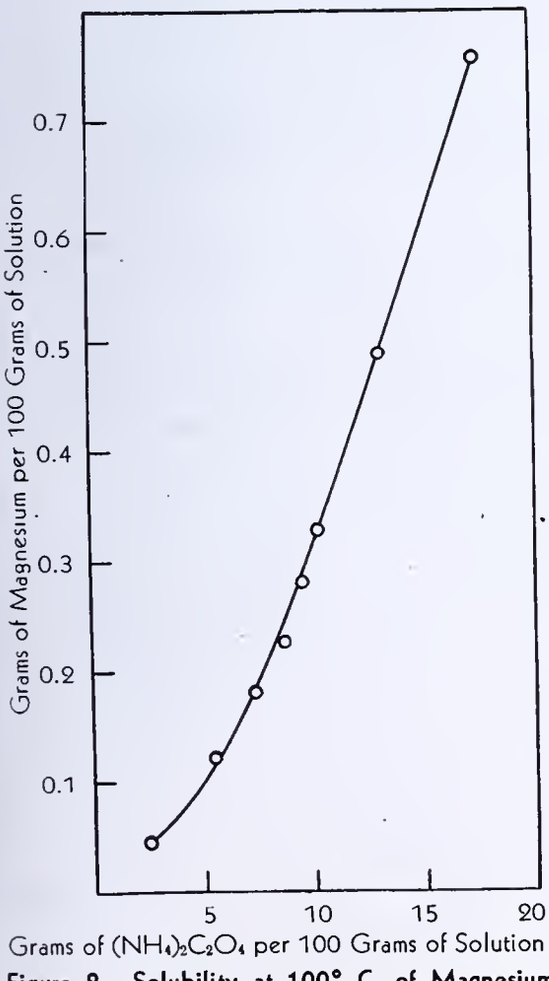

Figure 2. Solubility at 100° C. of Magnesium Oxalate in Ammonium Oxalate Solutions (2)

Table II. Quantity of Ammonium Oxalate Required

Magnesium	$(NH_4)_2C_2O_4 \cdot H_2O$	Calcium Carbonate Found		Error, Ca	
		Taken	1st pptn.	2nd pptn.	1st pptn.	2nd pptn.
Grams/100 ml.	*at 90° C.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
0.046	3.0	50.3	50.7	49.9	+ 0.16	− 0.16
	3.0	50.3	50.5	49.7	+ 0.08	− 0.24
0.116	3.0	56.8	128.6	56.4	+28.7	− 0.16
	3.0	56.8	142.0	56.5	+34.1	− 0.12
	3.0	101.0	218.4	100.3	+47.0	− 0.28
	3.0	101.0	224.5	100.6	+49.4	− 0.16
	5.0	56.8	60.6	56.1	+ 1.5	− 0.28
	5.0	56.8	57.3	55.1	+ 0.2	− 0.28
	5.0	101.0	131.2	100.4	+12.1	− 0.24
	5.0	101.0	134.0	100.4	+13.2	− 0.24
	7.0	50.3	50.5	49.6	+ 0.08	− 0.28
	7.0	50.3	50.3	49.5	0.00	− 0.32
	7.0	101.0	101.5	100.1	+ 0.20	− 0.36
	7.0	101.0	101.5	100.2	+ 0.20	− 0.32
0.231	5.0	56.8	86.5	58.0	+11.9	+ 0.48
	5.0	56.8	228.5	98.1	+68.7	+16.5
	10.0	56.8	55.8	55.3	− 0.4	− 0.6
	10.0	56.8	55.7	55.2	− 0.4	− 0.7
	15.0	56.8	54.4	54.2	− 1.0	− 1.0
	15.0	56.8	54.0	54.1	− 1.1	− 1.1
0.462	5.0	51.3	Very high		Very high	
	10.0	51.3	712.2	238.4	+264	+ 75
	10.0	51.3	518.8	341.0	+187	+116
	15.0	51.3	106.9	50.5	+22.2	− 0.32
	15.0	51.3	91.7	49.0	+16.1	− 0.9
	20.0	51.3	48.7	48.7	− 1.0	− 1.0
	20.0	51.3	47.9	47.5	− 1.4	− 1.5
	20.0	68.8	65.5	64.7	− 1.3	− 1.6
	20.0	68.8	65.9	65.6	− 1.2	− 1.3
	25.0	51.3	45.8	45.4	− 2.2	− 2.3
	25.0	68.8	63.6	62.7	− 2.1	− 2.4
1.16	25.0	56.8	Very high		Very high	
	30.0	56.8	145.0	55.7	+35.3	− 0.4
	30.0	56.8	153.0	47.4	+38.5	− 3.8
	35.0	56.8	43.0	42.5	− 5.5	− 5.7
	35.0	56.8	42.3	42.2	− 5.8	− 5.8

Table III. Recommended Quantity of Ammonium Oxalate

Magnesium Present	$(NH_4)_2C_2O_4 \cdot H_2O$ Added	Solubility Correction, Ca
G./100 ml.	*G./100 ml.*	*Mg./100 ml.*
0.10	7	0.20
0.20	11	0.35
0.30	15	0.60
0.40	20	1.4
0.50	25	2.0

Table IV. Mode of Precipitation

Magnesium	$(NH_4)_2C_2O_4 \cdot H_2O$	Error in First Pptn. (as $CaCO_3$)			MgO in Ppt.		
		Method A	Method B	Method C	Method A	Method B	Method C
Grams/100 ml.		*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
0.075	1.5	+1.3	...	...	1.0	...	...
	3.0	...	+0.6	+0.6	...	0.5	0.7
	5.0	+0.7	+0.3	...	0.6	0.5	...
	6.0	...	...	−0.5	...	...	0.2
0.200	1.5	+4.7	...	...	3.3	...	...
	5.0	+2.4	+2.7	+86.7	1.3	...	...
	8.0	...	...	+0.5	...	...	0.5
	11.0	+1.4	−1.3	−0.6	0.6	0.4	0.2
0.400	10.0	+13.5	+5.3	...	...	...	...
	13.0	−1.1	...	+11.6	0.7	...	...
	15.0	−2.2	−2.6	−1.4	0.9	0.1	0.3
0.462	20.0	...	...	−3.8	...	...	0.1

Method A, Kolthoff and Sandell (*5*).
Method B, reverse order, addition of sample to hot $(NH_4)_2C_2O_4$ solution.
Method C, addition of weighed quantity of solid $(NH_4)_2C_2O_4 \cdot H_2O$ directly to hot sample.
All determinations made with calcium content of about 50 mg. of $CaCO_3$.
Figures listed are averages of duplicate determinations.

Mode of Precipitation. This is the point to which previous investigators have devoted most of their attention in an effort to improve the calcium-magnesium separation. It has been clearly shown (*1*, *3*, *5*, *6*, *7*) that in magnesium-calcium ratios (by weight) of 1 to 1 or lower a better separation is attained by slow neutralization of an acid oxalate solution than by addition of oxalate to a neutral solution. In this case, sufficient oxalate according to Figure 2 is not always added and it is necessary to depend upon supersaturation to keep magnesium oxalate from precipitating (*4*).

It was pointed out by Blasdale (*1*) that there is no purpose in the slow neutralization of a sample containing much magnesium because the calcium does not begin to precipitate in this case until the solution is nearly neutral. This observation has been repeatedly confirmed; in fact, it was noted that the neutralization of a hot acid solution containing 20 grams of ammonium oxalate monohydrate and 0.4 gram of magnesium ion resulted sometimes in a heavy precipitation of magnesium oxalate which tended to dissolve as the neutralization proceeded. This behavior is to be expected, since in an acid solution the oxalate-ion concentration is low at first and as it increases during the neutralization a point is reached at which the oxalate-ion concentration is favorable for the precipitation of magnesium oxalate. As the oxalate-ion concentration continues to increase during the neutralization, the precipitated magnesium oxalate goes back into solution, presumably in the form of a complex ion. The exact nature of this complex ion is not clear, but it may contain a mole ratio of oxalate to magnesium as high as 4 to 1, since the mole ratios calculated from the solubilities shown in Figure 2 appear to approach this value as the magnesium content increases.

It was decided, therefore, to investigate three modes of precipitation according to the following scheme. Results are shown in Table IV.

Method A. According to Kolthoff and Sandell (*5*). This procedure consists of the dropwise addition of 6 *N* ammonia to an acid solution containing 1.5 grams or more of ammonium oxalate monohydrate per 100 ml. at 70° to 80° C. until the solution is basic to methyl red indicator. The solution is then allowed to stand without further heating for 1 hour and filtered cold. This procedure was modified in cases where more than 5.0 grams of ammonium oxalate monohydrate was used by maintaining the solution at 70° C. in order to prevent precipitation of ammonium oxalate monohydrate upon cooling.

Method B. Reverse order. The sample was added dropwise from a separatory funnel to a hot solution of ammonium oxalate of such dilution that the final volume would be 100 ml. The precipitate was digested 0.5 hour at 70° C. and filtered hot.

Method C. Solid ammonium oxalate monohydrate was added directly to the hot solution according to the method given under Procedure.

After the first precipitation was completed according to one of the above procedures, the ignited and weighed precipitate of calcium carbonate containing any coprecipitated magnesium was dissolved in hydrochloric acid, and a second precipitation was made (see Procedure). The filtrate from the second precipitation was examined for magnesium by the Titan yellow method (*8*) as a check on the loss in weight between the first and second precipitations.

Conclusions drawn from the results shown in Table IV may be summarized as follows:

Method A results in a somewhat greater coprecipitation of magnesium than method B or C, but gives less precipitation of magnesium oxalate where a deficiency of ammonium oxalate is used.

Method B is somewhat less convenient than method C and does not give results that are much, if any, better than those with method C.

If sufficient ammonium oxalate is present, methods B and C result in negligible coprecipitation of magnesium, about 0.1 to 0.3 mg. of magnesium usually being found with the ignited calcium carbonate. Results are low, however, because of the solubility of calcium oxalate in the concentrated ammonium oxalate solutions.

Method A lends itself to good accuracy without solubility correction in samples containing magnesium-calcium ratios by weight as high as 20 to 1 if 5.0 grams of ammonium oxalate monohydrate are used per 100 ml. of solution, a double precipitation is made, and not more than 0.2 gram of magnesium ion per 100 ml. is present.

The stability toward boiling of a magnesium solution containing excess oxalate was tested by boiling gently for 2 hours a solution containing 0.462 gram of magnesium ion, 20.0 grams of ammonium oxalate and 20.7 mg. of calcium ion. The precipitated calcium oxalate was then filtered, washed, ignited, and weighed in the regular manner. No evidence of postprecipitation of magnesium oxalate was found. This point was checked further by refluxing for 8 hours a pure magnesium chloride solution containing 0.462 gram of magnesium and 20.0 grams of ammonium oxalate per 100 ml. No visible precipitation of magnesium oxalate occurred.

Applications. Results which can be obtained by a single precipitation of calcium oxalate in the analysis of three samples containing a high magnesium-calcium ratio are shown in Table V. While these results are in general satisfactory, the method is not

Table V. Miscellaneous Applications

Description	Ratio by Weight Mg:Ca	Correction Applied, Ca	Magnesium Present	Volume	$(NH_4)_2C_2O_4 \cdot H_2O$ Used	Calcium Present	Calcium Found $CaCO_3$[a] method	Calcium Found KMnO method
		Mg.	*Grams*	*Ml.*	*Grams*	%	%	%
Dilute (15%) $MgCl_2$ liquor	16:1	1.4	0.43	100	20	0.232[b]	0.231	0.229
							0.231	0.229
							0.232	0.229
Concentrated (34%) $MgCl_2$ liquor	85:1	4.0	1.08	200	50	0.099[b]	0.098	...
							0.097	...
							0.096	...
B. of S. burned magnesite (standard sample 104)	22:1	1.4	0.41	100	20	2.39	2.39	...
							2.36	...
							2.37	...

[a] By method shown under procedure, using single oxalate precipitation.
[b] By removal of 90% of magnesium with NaOH, followed by double oxalate precipitation of calcium, then ignition to $CaCO_3$.

ways practical because of the large quantity of ammonium alate required, and the necessity for applying an accurately own empirical correction due to solubility of calcium oxalate. he calcium oxalate precipitate can be titrated with permanga- te with results that check closely those obtained by weighing calcium carbonate, as shown in Table V.

CONCLUSIONS

The separation of calcium by precipitation as oxalate from lutions containing much magnesium is limited by the solubility calcium oxalate, which becomes appreciable in hot concen- ated ammonium oxalate solutions, and by the necessity of ing large amounts of ammonium oxalate in order to prevent ecipitation of magnesium oxalate. By the addition of suf- ient ammonium oxalate, up to 0.5 gram of magnesium ion can held in solution in 100-ml. volume at 80° C.

Coprecipitation of magnesium is small if a large excess of mmonium oxalate is added directly to a hot neutral solution ntaining magnesium and calcium.

In the range of magnesium-calcium ratios (by weight) of 20 to 1, calcium can be determined with acceptable accuracy by a single precipitation of calcium oxalate using a large amount of ammonium oxalate and an empirical correction for solubility of calcium oxalate, or by double precipitation using not over 5 grams of ammonium oxalate per 100 ml. as precipitant.

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Systematic Polarographic Metal Analysis

Determination of Tin, Lead, Nickel, and Zinc in Copper-Base Alloys

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rocedures are described for determining tin, lead, nickel, and zinc copper-base alloys. Other minor elements such as antimony, ismuth, iron, and manganese, do not interfere, and two samples iffice for all four determinations. Electrolysis at controlled poten- al is used to remove copper (and antimony, bismuth, etc.) prior the determination of lead and tin, and to remove copper, lead, d tin prior to the determination of nickel and zinc. Separate iquot portions of the solution remaining after removal of copper e used for polarographic determination of lead and tin, the former in 1 *N* sodium hydroxide, and the latter in a supporting electrolyte composed of 4 *M* ammonium chloride and 1 *N* hydrochloric acid. Nickel and zinc are determined simultaneously in the solution remaining after removal of copper, lead, and tin; an ammoniacal supporting electrolyte is used, and small amounts of nickel may be determined accurately in the presence of large amounts of zinc. All four determinations can be completed in about 2.5 hours. Results obtained with several representative Bureau of Standards copper-base alloys agreed very well with accepted values.

PROCEDURES for the polarographic determination of copper, zinc, lead, iron, and nickel in brass have been described y Hohn (*2*, *3*) and Mnich (*3*, *11*), a method of determining only pper and zinc in such alloys has been discussed by Tyler and rown (*16*), and a method for nickel in brasses and bronzes was de- eloped by Milner (*10*). Several studies of the polarographic char- cteristics of tin have been made (*4*, *8*, *9*, *13*, *14*, *15*), but no ethod for the polarographic determination of this element in lloys has previously been described. In this paper a systematic rocedure for the determination of tin, lead, nickel, and zinc in arious kinds of copper-base alloys is presented. Other minor lements which may be present in such alloys, such as antimony, ismuth, iron, and manganese, do not interfere, and two samples uffice for all four determinations.

Since the wave of cupric copper precedes that of the other ele- ents concerned, it must be removed. Taking advantage of the act that the cuprous cyanide complex ion is not reducible at the ropping electrode in basic solution, Hohn (*2*) and Milner (*10*) ecommended the reduction of cupric copper by cyanide ion in mmoniacal medium to eliminate its interference in the deter- ination of nickel. This procedure is not applicable when tin nd lead are also to be determined, and Milner (*10*) found that he diffusion current of the nickel cyanide complex was influenced y the amount of zinc present.

In the present method copper is removed by electrolysis with a latinum cathode at controlled potential, and lead and tin are directly determinable in the residual solution in the presence of nickel and zinc. Antimony and bismuth are removed with the copper, and iron is finally present in the ferrous state and does not interfere. Nickel and zinc are determined in a second sample after removal of copper, tin, and lead (and antimony, bismuth, etc.) by controlled potential electrolysis. Although, on paper, it might seem that a precipitation method of removing copper would be simpler than controlled potential electrolysis because of the large amount of precipitate that would have to be handled, electrolytic separation is more convenient. Using the automatic apparatus previously described (*6*, *7*), electrolytic separation actually requires less of the operator's time than that involved in processing a precipitate. Furthermore, in a precipitation method errors due to coprecipitation must be considered, and the necessity of introducing relatively large quantities of reagents frequently complicates subsequent polarographic determinations. In electrolytic separations only hydrogen ion in amount equivalent to the metal deposited is introduced, and the preparation of the residual solution for polarographic determinations is thus facilitated.

The solution remaining after removal of copper, which contains the tin in the stannic state, is diluted to a known volume and separate aliquot portions are used for the determination of lead and tin. Nickel and zinc are determined simultaneously in the solution remaining after electrolytic removal of copper, tin, and lead from a second sample. In the ammoniacal sup-

porting electrolyte employed the wave of nickel precedes, and is well separated from, the wave of zinc, which permits the simultaneous determination of the two metals from a single polarogram (Figure 2). The amount of nickel in a copper-base alloy is usually considerably less than the amount of zinc, and accurate determinations of nickel may be made in the presence of a large excess of zinc without difficulty.

EXPERIMENTAL TECHNIQUE

The electrolytic separations were performed with a platinum gauze cathode 5 cm. high and 5 cm. in diameter, and a platinum gauze anode 5 cm. high and 2.5 cm. in diameter which was mounted inside and coaxially with the cathode. Efficient stirring was provided by a magnetic stirrer consisting of a bar of soft iron sealed in a Pyrex glass shell activated by a rotating magnet mounted on the end of a motor shaft below the 250-cc. beaker which served as the electrolysis cell. (This device is obtainable from A. H. Thomas Co., Philadelphia.) A saturated calomel electrode was used to control the cathode potential, and the tip of the salt bridge was placed as close as possible to the outside surface of the cathode near its middle.

The electrolyses were performed by so adjusting the total e.m.f. applied to the cell that the potential of the cathode versus the saturated calomel electrode attained the desired value, which was maintained as electrolysis proceeded by suitable readjustment (usually decrease) of the applied e.m.f. The electrical circuit used which has been described (*6*, *7*), performs this function automatically and maintains the cathode potential to within ±0.02 volt of any desired value without any attention. Not more than 5 minutes of the operator's time is required for an electrolytic separation with this automatic apparatus, and the total elapsed time is usually less than 45 minutes. A manually operated circuit of the type described by Sand (*12*) can also be used, but it requires constant attention.

When the electrolysis was complete (5 to 10 minutes after the current fell to a small constant value) the stirrer was stopped, the beaker was lowered from the electrodes with the circuit still connected, and the electrodes were washed with a small amount of water from a wash bottle. If lead has been deposited on the cathode the washing should be done quickly with a minimal amount of water, because appreciable amounts of lead are dissolved by prolonged washing (*7*). Since no more than 2 or 3 cc. of the original 200 cc. of solution electrolyzed adheres to the electrodes, and since the accuracy of the subsequent polarographic analysis is of the order of 1%, a single washing of the electrode suffices. The combined residual solution and washings were prepared for polarographic analysis as described below under the separate determinations.

Polarograms were recorded with a Sargent-Heyrovský Model XI polarograph, whose recording galvanometer was calibrated as previously described (*3*). The polarographic cell was placed in a water thermostat at 25° C.

The dropping electrode assembly, including a stop-clock device which automatically measures the rate of flow of mercury, has been described (*5*). Preliminary measurements, in which accurate values of half-wave potentials were desired, were made with the usual H-type cell with saturated calomel anode (*3*). In subsequent determinations of nickel and zinc in an ammoniacal supporting electrolyte a length of lead wire (No. 18) wrapped in the form of a helix directly on the dropping electrode capillary served as anode. The potential of this lead anode in a solution containing 0.2 *M* ammonium ion, 1 *M* ammonia, and 0.1 *M* sulfite ion is about −0.68 volt *vs.* the S.C.E. The anode reaction is $Pb + 2OH^- = Pb(OH)_2 + 2e$, and since lead hydroxide is very insoluble in ammoniacal media no appreciable concentration of lead ion is produced in the solution.

For determination of tin in a supporting electrolyte containing 4 *M* ammonium chloride and 1 *M* hydrochloric acid, and for determination of lead in 1 *M* sodium hydroxide, a silver wire anode wrapped in a helix on the dropping electrode was used (*5*). In 4 *M* ammonium chloride–1 *M* hydrochloric acid the potential of the silver anode is approximately −0.1 volt, and in 1 *M* sodium hydroxide −0.2 volt, *vs.* the saturated calomel electrode.

In the determination of nickel and zinc in ammoniacal medium, sulfite ion (0.1 *M*) was added to the solutions to remove oxygen. In addition to its convenience, sulfite ion removes oxygen more effectively than nitrogen or hydrogen gas. In these determinations a small (10- to 25-cc.) Erlenmeyer flask served as the polarographic cell. The use of sulfite to remove oxygen, and of the lead or silver wire anodes, constitutes the simplest possible technique, and it is very well suited to rapid routine analysis.

Concentrations were computed from the relation $C = i_d/Im^{2/3}t^{1/6}$ millimoles per liter, where i_d is the observed diffusion current (microamperes), I is the diffusion current constant, m is the rate of flow of mercury (mg. per second), and t is the drop time (*4*). The use of this relation, with previously determined values of diffusion current constants, eliminates the calibration of each dropping electrode with known concentrations of the substance being determined. The pertinent diffusion current constants at 25° C. determined in this investigation are summarized in Table I. In using these data it should be borne in mind that the above relation fails when the drop time is less than about 1 second.

LEAD AND TIN

A suitable size of sample is dissolved in hydrochloric acid with the aid of the minimal amount of nitric acid required to effect complete solution. The solution is diluted and treated with hydrazine dihydrochloride as an anodic depolarizer, and the copper (and antimony) is removed by electrolysis with the potential of the platinum cathode maintained constant at −0.3 volt *vs.* the saturated calomel electrode. In the deposition of 1 gram of copper from a volume of about 200 cc. the initial current was usually about 8 amperes, and it decreased to about 0.0 ampere within about 45 minutes. In agreement with the experience of Diehl and Brouns (*1*) it was found that the deposition of metallic copper on the cathode proceeds slowly at first, and during the initial stage of electrolysis reduction of cupric ion to a chloride complex of the cuprous state appears to be the predominant cathode reaction. Reduction to the cuprous state is almost complete after a few minutes and deposition of metallic copper then proceeds rapidly to completion. Diehl and Brouns (*1*) recommended the use of a 1 to 1.5 *M* hydrochloric acid solution for the separation of copper from tin, but the writer obtained much better deposition when the concentration of hydrochloric acid was kept just large enough to prevent hydrolysis of stannic ion, not over 0.5 *M*. With this minimal concentration of hydrochloric acid the complications observed by Diehl and Brouns (*1*) in more concentrated chloride solution were not encountered, the presence or absence of tin was immaterial; and antimony was completely deposited with the copper. The potential should not be allowed to exceed about −0.4 volts, as otherwise lead and tin may deposit, and it should be at least −0.3 volt to obtain rapid deposition of copper. The solution remaining after the electrolysis, which contains the tin in the stannic state, is diluted to a known volume and separate aliquot portions are taken for the determination of lead and tin.

Lead is determined in 1 *M* sodium hydroxide as supporting electrolyte; in this medium the half-wave potential of the plumbite ion is −0.76 volt *vs.* the saturated calomel electrode and the wave characteristics are excellent (*4*). Since stannic tin does not produce a wave in this solution (*4*), nickel and iron are precipitated, and the half-wave potential of the zincate ion is −1.50 volt.

Table I. Pertinent Half-Wave Potentials and Diffusion Current Constants

Temperature = 25° C. The half-wave potentials are referred to the saturated calomel electrode. The diffusion current constants correspond to the observed diffusion current in microamperes, when the concentration is 1 millimolar, and when the value of $m^{2/3}t^{1/6}$ is 1 mg.$^{2/3}$ sec.$^{-1/2}$. All solutions contained 0.005% gelatin as a maximum suppressor.

Metal	Supporting Electrolyte	$E_{1/2}$ Volts	I
Lead	1 *M* NaOH	−0.76	3.40
	4 *M* NH_4Cl + 1 *M* HCl	−0.52	3.52
Tin	4 *M* NH_4Cl + 1 *M* HCl	−0.25	2.84[a]
		−0.52	3.49[b]
			6.33[c]
Nickel	0.2 *M* NH_4Cl + 1 *M* NH_3	−1.06	3.54
	1 *M* NH_4Cl + 1 *M* NH_3	−1.09	3.56
	3 *M* NH_4Cl + 1 *M* NH_3	−1.12	3.33
Zinc	0.2 *M* NH_4Cl + 1 *M* NH_3	−1.33	3.78
	1 *M* NH_4Cl + 1 *M* NH_3	−1.33	3.82
	3 *M* NH_4Cl + 1 *M* NH_3	−1.36	3.94

[a] First wave.
[b] Second wave.
[c] Total double wave.

ry small amounts of lead can be determined without interrence from the other metals. The amounts of nickel and iron esent in copper-base alloys are relatively small, and usually the droxides of these metals need not be filtered off. Curiously ough it was found that sulfite (0.1 *M*) does not remove oxygen iciently from sodium hydroxide solutions that contain lead; parently the plumbite ion inhibits the oxygen-sulfite reaction, d hence nitrogen or hydrogen must be used.

The polarogram of this solution also includes the wave of ncate ion at −1.50 volt *vs.* the saturated calomel electrode, d, provided the amount of lead does not exceed the amount of nc, this wave might be used for the determination of zinc. bwever, the characteristics of the zincate wave leave much to desired and the writer prefers the determination of zinc in mmoniacal medium in conjunction with the nickel determination.

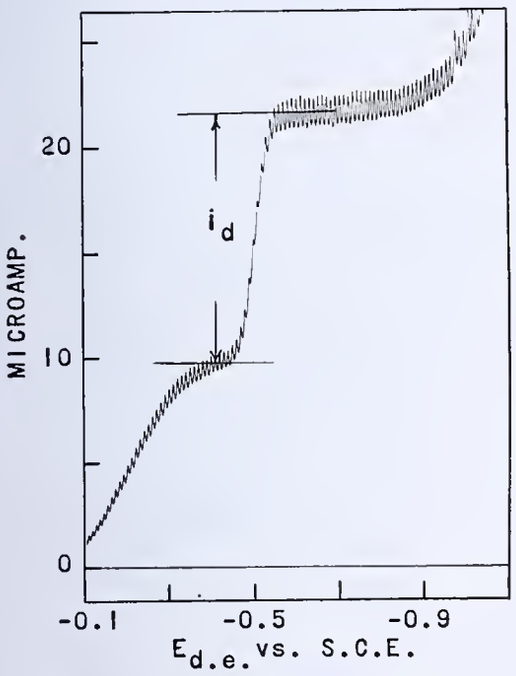

Figure 1. Typical Polarogram of 2.08 Millimolar Stannic Tin in 4 M Ammonium Chloride, 1 M Hydrochloric Acid, and 0.005 % Gelatin, Showing Method of Measuring Diffusion Current

Tin is determined in a supporting electrolyte containing 4 *M* mmonium chloride and 1 *M* hydrochloric acid. In this medium annic tin produces a double wave, corresponding to stepwise duction first to the stannous state and then to the metal, with lf-wave potentials of −0.25 and −0.52 volt *vs.* the saturated lomel electrode (*9*). The first diffusion current is not completely developed before the second wave begins, but the total ffusion current is very well defined (see Figure 1). The height the first wave is 45% that of the total double wave, instead of % as it would be if the diffusion current were completely deloped, but this creates no difficulty because the proportion is nstant regardless of the concentration of the tin. Hence her the first wave, the second wave alone, or the total double ve may be measured; the diffusion current constants, $i_d/m^{2/3}t^{1/6}$), at 25° are listed in Table I. Since the measurement either the first wave or total wave requires correction for the sidual current, which must be separately determined because e first wave starts from zero applied e.m.f., the writer prefers easurement of the second wave. The half-wave potential of d in this supporting electrolyte is virtually coincident with that the second stage of the reduction of the chlorostannate ion, d hence the second wave includes a diffusion current due to lead which correction must be applied. As the amount of lead in copper-base alloy seldom exceeds the amount of tin, and usually is less, such a correction is not as objectionable as it might otherwise be.

The method of applying this correction is best illustrated by a typical example. Aliquot samples (50 cc.) were used for each determination and in each case were finally diluted to 100 cc. in the appropriate supporting electrolyte. The observed diffusion current of lead in 1 *N* sodium hydroxide was 2.34 microamperes with $m^{2/3}t^{1/6} = 1.57$, and the diffusion current of the second wave in 4 *M* ammonium chloride–1 *M* hydrochloric acid was 7.10 microamperes with $m^{2/3}t^{1/6} = 1.60$. From the data in Table I the diffusion current constant of lead in 4 *M* ammonium chloride–1 *M* hydrochloric acid is seen to be somewhat larger than in 1 *M* sodium hydroxide, the ratio being 3.52/3.40 = 1.036. The contribution of lead to the second wave in the former medium was thus 2.34 × 1.036 × 1.60/1.57 = 2.47 microamperes, and the diffusion current due to tin was 7.10 − 2.47 = 4.63 microamperes. The concentration of lead was 2.34/(3.40 × 1.57) = 0.438 millimolar, and that of tin was 4.63/(3.49 × 1.60) = 0.829 millimolar.

For percentages of tin and lead between about 0.1 and 10, a 0.5- to 1-gram sample is suitable, and it is convenient to dilute the residual solution after removal of the copper to 250 cc. and take 50-cc. aliquots for the polarographic determinations.

Procedure. Dissolve a 0.5- to 1-gram sample of the alloy in a warm mixture of 6 cc. of 12 *N* hydrochloric acid, 4 cc. of water, and 1 cc. of concentrated nitric acid in a Kjeldahl flask. Dilute to about 50 cc. and boil briefly to remove oxides of nitrogen and chlorine. Transfer the solution to a 250-cc. beaker, add 2 grams of pure hydrazine dihydrochloride, dilute to about 200 cc., and electrolyze with the potential of the platinum cathode at −0.35 volt *vs.* the saturated calomel electrode. Transfer the residual solution to a 250-cc. volumetric flask, cool to room temperature, and dilute to the mark. The final solution will be about 0.4 *N* in respect to hydrochloric acid.

Transfer a 50-cc. aliquot (corresponding to one fifth of the sample) to a 100-cc. volumetric flask, add 24 cc. of 5 *N* sodium hydroxide (or 4.8 grams, 0.12 mole, of the solid) and 2.5 cc. of 0.2% gelatin solution, and dilute to the mark. Transfer a portion of the solution to the polarographic cell in a water thermostat at 25.0° ± 0.2° C., remove dissolved air with nitrogen or hydrogen, record the polarogram, and measure the diffusion current of the lead. Determine the *m*-value of the dropping electrode, measure the drop time at the potential at which the diffusion current was measured, and compute the concentration of lead from the relation $C = i_d/3.40\ m^{2/3}t^{1/6}$ (see Table I).

Transfer another 50-cc. aliquot to a 100-cc. volumetric flask, add 21 grams (0.4 mole) of solid ammonium chloride, 6.6 cc. (0.08 mole) of 12 *N* hydrochloric acid, and 2.5 cc. of 0.2% gelatin solution, and dilute to about 90 cc. Shake until the ammonium chloride is dissolved, warm back to room temperature, and dilute to the mark. Transfer a portion of the solution to the polarographic cell in a water thermostat at 25.0° ± 0.2° C., remove dissolved air with nitrogen or hydrogen, record the polarogram, and measure the diffusion current of the second wave (see Figure 1). Apply the correction for lead, and compute the concentration of tin, as described above.

The concentrations of the components of the supporting electrolytes are not critical, but should be within about 10% of the values specified.

NICKEL AND ZINC

A sample of the alloy is dissolved, and the copper is removed electrolytically exactly as in the determination of lead and tin. After most of the copper has deposited (about 15 minutes), the potential is increased to −0.70 volt *vs.* the saturated calomel electrode to deposit tin and lead. The initial current at −0.70 volt was usually of the order of 3 to 5 amperes, and ordinarily, it decreased to a constant value of about 1 ampere after about 15 minutes. The current at −0.70 volt does not decrease to zero because hydrogen ion is reduced at this potential. To ensure complete deposition of tin and lead the electrolysis should be continued for about 10 minutes after the current decreases to a constant value. Since tin and lead are precipitated in the ammoniacal supporting electrolyte used for the determination of nickel and zinc it is not essential that they be removed completely; in fact, if the amount of tin and lead present does not greatly exceed the amount of nickel or zinc (whichever is the smaller), they need not be removed at all. A large precipitate

of the hydrous oxides of tin and lead is objectionable because it may coprecipitate significant amounts of nickel and zinc.

A systematic study was made of the influence of the concentration of ammonium chloride and ammonia on the nickel and zinc waves; the effect of various concentrations of ammonium chloride at a constant concentration (1 *M*) of ammonia is shown by the data in Table I. Increasing the concentration of ammonium chloride shifts the half-wave potentials of both metals to slightly more negative values, and since the half-wave potential of nickel is shifted more than that of zinc, the separation of the waves is less satisfactory in 3 *M* ammonium chloride than in 0.2 *M*. Even very large changes in the concentration of the ammonium chloride produce relatively small changes in the diffusion current constants, so that the adjustment of the concentration of ammonium chloride is not at all critical. Increasing the concentration of ammonia from 0.1 to 5 *M* at a constant concentration (0.2 *M*) of ammonium chloride also decreases the separation of the waves, but causes only slight changes in the diffusion current constants. The optimum supporting electrolyte is one that contains about 0.2 *M* ammonium chloride and 1 *M* ammonia; in this medium the two waves are so well separated that there is no difficulty in determining nickel in the presence of a fiftyfold or even larger excess of zinc (see Figure 2).

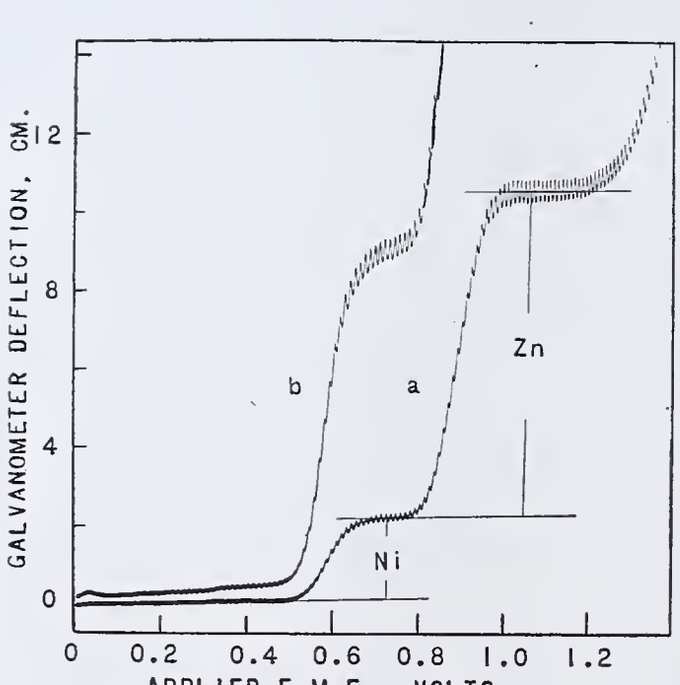

Figure 2. Polarograms of Nickel and Zinc

Obtained in analysis of Bureau of Standards cast bronze No. 52b, containing 0.72% nickel and 2.96% zinc. Supporting electrolyte contained 0.2 *M* ammonium chloride, 1 *M* ammonia, 0.1 *M* sodium sulfite, and 0.005% gelatin. A lead wire anode was used. Curve *a* was recorded with a galvanometer sensitivity of 0.748 microampere per cm., and curve *b* with a sensitivity of 0.187 microampere per cm. to magnify the nickel wave.

PROCEDURE. Dissolve a 0.5- to 1-gram sample, and prepare the solution for electrolysis, exactly as for determination of lead and tin. Electrolyze first at a potential of −0.35 volt *vs.* the saturated calomel electrode, and after most of the copper has deposited increase the potential to −0.70 volt to deposit tin and lead. Continue the electrolysis for about 10 minutes after the current decreases to a constant value. Transfer the residual solution to a 250-cc. volumetric flask and dilute to the mark.

Transfer a 50-cc. aliquot to a 100-cc. volumetric flask, add 8 cc. of 15 *M* ammonia, 1 to 1.5 grams (approximately 0.01 mole) of pure anhydrous sodium sulfite to remove oxygen, and 2.5 cc. of 0.2% gelatin, and dilute to the mark. Place a portion of the solution in a polarographic cell in a water thermostat at 25.0° ± 0.2° C.—e.g., a 15-cc. Erlenmeyer flask if a lead wire anode wrapped on the dropping electrode is used—and record the polarogram. If the amount of nickel is much smaller than that of zinc, a second polarogram should be recorded at an increased sensitivity to magnify the nickel wave to a value large enough for accurate measurement. Measure the two diffusion currents (see Figure 2), the *m*-value of the capillary, and the drop times at the potentials at which the diffusion currents were measured. Compute the concentration of nickel from the relation $C = i_d/3.54\,m^{2/3}\,t^{1/6}$, and the concentration of zinc from the relation $C = i_d/3.78\,m^{2/3}\,t^{1/6}$.

Polarograms of some samples showed a final current increase at about −1.5 volts, which interfered with the full development of the diffusion current of the zinc. This was usually observed with samples that contained relatively large amounts of iron. In the presence of hydrazine the iron in the electrolyzed solution will be present in the ferrous state, and from the work of Voriskova (*17*) it is known that ferrous iron produces a wave in ammoniacal solutions whose half-wave potential is −1.49 volts *vs.* the saturated calomel electrode. The interference can easily be eliminated by treating the electrolyzed solution, or an aliquot of it, with 5 cc. of concentrated nitric acid, and evaporating to dryness to oxidize ferrous iron and hydrazine. The residue is taken up in an appropriate amount of dilute hydrochloric acid and the solution is prepared for analysis as above.

Table II. Analysis of Bureau of Standards Copper-Base Alloys

Sample and Bureau of Standards Analyses	Polarographic Analyses, % Pb	Sn	Ni	Zn
Phosphor bronze 63a Cu 78.48, Pb 8.92, Sn 9.76, Ni 0.32, Zn 0.61, Sb 0.49, Fe 0.52, P 0.58, S 0.11	9.04	9.84	0.32	0.60
Cast bronze 52b Cu 88.26, Pb 0.01, Sn 8.00, Ni 0.72, Zn 2.96, Fe 0.034	0.02	7.82	0.70	2.96
Ounce metal 124a Cu 85.05, Pb 4.86, Sn 4.81, Ni 0.001 Zn 5.25, Fe 0.004	4.84	5.00	..	5.29
Brass 37c Cu 70.07, Pb 0.97, Sn 0.96, Ni 0.58, Zn 27.22, Fe 0.17	0.92	0.97	0.59	27.8

RESULTS AND DISCUSSION

The foregoing procedures were tested with several representative Bureau of Standards copper-base alloys, with the results shown in Table II.

With percentages of the various metals smaller than about 5% the polarographic analyses agree with the bureau's values about as closely as the latter are known, and even with larger percentages the agreement is satisfactory. For determining fractions of a per cent of these metals, especially zinc, the polarographic method is probably more reliable than the classical procedures, but it cannot compete with the classical methods, except from the standpoint of convenience, for amounts larger than about 10%. Determinations of the four metals can be completed within about 2.5 hours by the polarographic procedure.

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Calculation of Specific Dispersion of Pure Hydrocarbons and Petroleum Fractions

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An equation is presented for the calculation of specific dispersion of pure hydrocarbons and petroleum fractions from common physical properties, density, refractive index, and approximate molecular weight, which can be estimated from mid-boiling point and density or viscosities at 100° and 210° F. This makes it possible to obtain specific dispersion on a highly colored sample or in a laboratory where no instrument is available for the direct measurement of dispersion. The accuracy of the calculated specific dispersion for petroleum fractions is comparable with the accuracy of measurements obtained on the Abbe instrument, ± 3 units.

THIS paper continues the general investigation in this laboratory of the interrelationship of physical properties and the relation of these properties to hydrocarbon structure (*4, 6–12, 15, 18*), and is particularly concerned with the calculation of specific dispersion of hydrocarbons from easily determined physical properties. This permits the use of specific dispersion by many laboratories which do not have equipment for experimentally determining specific dispersion, and extends the use of specific dispersion to highly colored samples on which it is difficult or impossible to obtain a direct measurement.

Specific dispersion is the difference between the refractive indices of a sample at two different wave lengths of light divided by the density of the sample. Most literature data are reported in terms of the *F* and *C* lines of hydrogen, although experimentally it is often more convenient to use other wave length combinations (*16*). In this paper specific dispersion, δ, is defined as:

$$\delta = \frac{n_F - n_C}{d} \times 10^4 \qquad (1)$$

where n_F = refractive index for the *F* line of hydrogen at 20° C.
n_C = refractive index for the *C* line of hydrogen at 20° C.
d = density at 20° C.

For hydrocarbons specific dispersion is a unique physical property because it is nearly constant for paraffins and naphthenes and is variable with the number of double bonds and their position in an unsaturated hydrocarbon molecule (*2, 3, 17*). Accurate determination of specific dispersion requires considerable skill and precision apparatus. Moreover, determination of this property on samples which are highly colored is very difficult and often impossible with the instruments available.

The application of specific dispersion to analysis of hydrocarbons has been seriously handicapped by the difficulty in obtaining this measurement. Early investigators attempted to develop atomic constants for the calculation of specific dispersion. More recently Thorpe and Larsen (*17*) have developed a method dependent on a knowledge of structure which limits the calculation to pure hydrocarbons whose structure is known. No correlation has been developed which permits calculation of specific dispersion for petroleum fractions.

In an effort to correlate the physical properties of hydrocarbons, Lipkin and Martin (*11*) have recently developed Equation 2 for the calculation of the sodium *D* refractive index of paraffins and naphthenes at 20° C.:

$$n_{\text{calcd.}} = \frac{69.878d - 0.4044Ad - 0.797A + 136.566}{5.543d - 0.746A + 126.683} \qquad (2)$$

where

$A = -10^5 \times$ the temperature coefficient of density, which is obtained from the approximate molecular weight, Table I (*12*). Molecular weight is obtained from boiling point and density (*15*) or the kinematic viscosity at 100° and 210° F. (*4*).

If double bonds are present, the calculated refractive index will be lower than the observed refractive index and the difference is a linear function of the specific dispersion. The method has the advantage over earlier methods of being applicable to petroleum fractions as well as pure hydrocarbons.

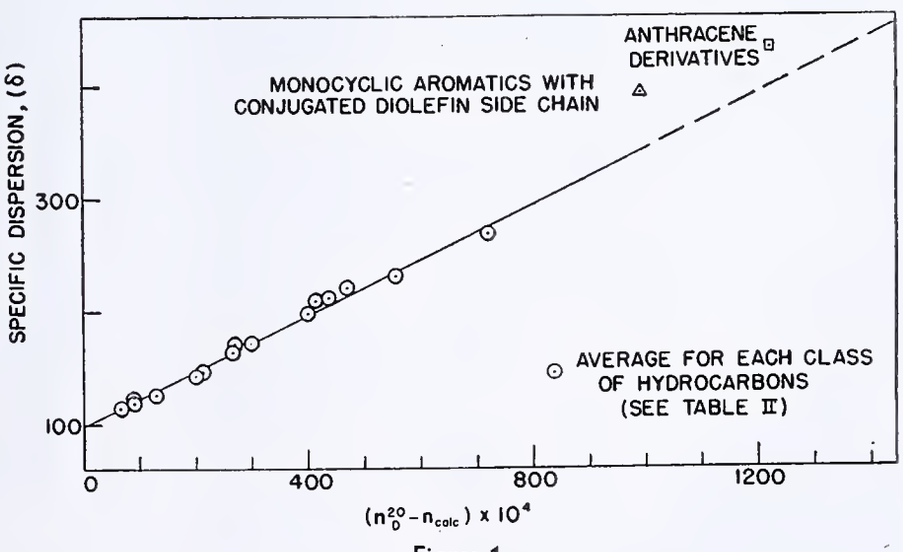

Figure 1

Table I. Effect of Unlike Additivities of Physical Properties on Calculation of Specific Dispersion

	% Saturate Volume	% Saturate Weight	50% B.P., °C.	d_4^{20}	n_D^{20}	δ	Mol. Wt. from B.P.–d	$10^4 \times n - n_{calcd.}$	$\delta_{calcd.}$	$\delta_{calcd.} - \delta$	δ_f[a]	$\delta_{calcd.} - \delta_f$
Saturate	100	100	113	0.7069	1.3988	97.9	113	1	98.5	0.6		
Blend												
1	80.0	76.6	116	0.7376	1.4171	114.4	112	59	112.8	−1.6	112.1	0.7
2	60.0	55.2	123	0.7687	1.4357	130.2	112	117	127.0	−3.2	126.4	0.6
3	40.0	35.3	130	0.7993	1.4540	144.3	112	174	140.9	−3.4	140.6	0.3
4	20.0	17.0	139	0.8312	1.4733	157.2	113	235	155.9	−1.3	154.9	1.0
Aromatic	0	0	148	0.8629	1.4923	169.1	115	293	170.1	1.0		

[a] Fictitious value calculated from δ of saturate and δ of aromatic assuming volume % additivity.

Table II. Agreement of Pure Compound Data

(Averaged by Group)

	No. of Compounds	$10^4 \times (n - n_{calcd.})$	$\delta_{calcd.}$	δ	$\delta_{calcd.} - \delta$
Noncyclics					
Olefins	120	86	119	122	− 3
Diolefins, conjugated	21	469	213	219	− 6
Diolefins, nonconjugated	8	198	147	142	5
Triolefins	3	411	199	209	−10
Acetylenes	9	64	114	113	1
Monocyclics					
Aromatics	47	264	163	163	0
Aromatics + olefin side chain	11	436	205	210	− 5
Naphthenes + olefin side chain	5	126	129	126	3
Cyclo-olefins	41	88	120	118	2
Cyclo-olefins + olefin side chain	5	210	150	146	4
Cyclodiolefins	12	266	164	171	− 7
Cyclodiolefins + olefin side chain	11	394	195	197	− 2
Polycyclics					
Alkyl naphthalenes	20	553	234	231	3
Miscellaneous dicyclics	32	296	171	170	1
Miscellaneous tricyclics	8	718	274	266	8
		Average deviation per compound			7
		Average deviation per group			4
		Deviation of average			0
Not averaged					
Aromatics + conjugated diolefin side chain	4	992	345	393	−48
Anthracenes	5	1373	435	506	−71

DERIVATION OF EQUATION

The difference between the observed refractive index and the refractive index calculated by Equation 2 was plotted against specific dispersion, as shown in Figure 1, for all olefinic, acetylenic, and aromatic type pure hydrocarbons on which specific dispersion data are reported. A best straight line was drawn originating at 98.3 specific dispersion for zero difference in refractive index. The value 98.3 is considered to be the average specific dispersion of paraffin and naphthene hydrocarbons (*2*, *17*).

From this line the following equation was derived for the calculation of specific dispersion:

$$\delta_{calcd.} = 98.3 + 2450\,(n - n_{calcd.}) \quad (3)$$

where

n = refractive index for the D line of sodium at 20°

and

$n_{calcd.}$ = value calculated by use of Equation 2

For pure hydrocarbons and mixtures of hydrocarbons of nearly the same specific dispersion, such as paraffin-naphthene mixtures or most aromatic extracts, this equation is satisfactory. If $(n - n_{calcd.})$ is negative the sample can be considered to consist of saturated hydrocarbons and may be assigned a δ of 98.3.

For mixtures such as ordinary petroleum fractions containing hydrocarbons of appreciably different specific dispersions, Equation 3 will give low results and is modified to include a small additive correction:

$$\delta_{calcd.} = 98.3 + 2450\,(n - n_{calcd.}) + C \quad (4)$$

where C = a small correction term roughly dependent upon th average molecular weight of the mixture. The following tab of approximate corrections, derived from the data in Table I may be used:

Correction to Be Used for Petroleum Fractions

Molecular Weight	C
100	2
200	5
300	7
400	8

Low results for mixtures are due to the fact that the physic properties used in this calculation and the property calculat are not additive on the same basis. Molecular weight is additi on a mole basis and density on a volume basis in the absence volume change on mixing. Refractive index is more near additive on a volume basis and specific dispersion more near additive on a weight basis.

Table I demonstrates this effect of difference in additivities properties. In this example the authors have blended in vario proportions an aromatic stock and a paraffin-naphthene sto of approximately equal molecular weight. When blended the stocks show less than 0.2% volume change on mixing, which is negligible factor in these calculations. Specific dispersions of t blends calculated by Equation 3 are lower than the experiment specific dispersions, but agree well with fictitious values calc lated on a volume basis from the specific dispersions of the para fin-naphthene stock and the aromatic stock. In most petroleu fractions the situation is further aggravated by the fact that t paraffin-naphthene component is significantly higher in molec lar weight than the aromatic component.

It is emphasized that the correction term is only approxima and intended for the types of hydrocarbon fractions normal

Table III. Agreement of Aromatic Extracts, Pulfrich Refractometer Data

	Boiling Range, °C.	50% B.P., °C.	d_4^{20}	n_D^{20}	Viscosity, Centistokes 100° F.	Viscosity, Centistokes 210° F.	δ	$10^4 \times (n - n_{calcd.})$	$\delta_{calcd.} - \delta$
6c straight run fraction	10	132	0.8611	1.4942	...	...	178	339	3
d	2	140	0.8649	1.4969	...	...	180	342	2
e	15	145	0.8683	1.4984	...	...	178	338	3
f	10	162	0.8652	1.4964	...	...	173	314	2
g	4	168	0.8695	1.5000	...	...	174	328	5
h	7	173	0.8748	1.5029	...	...	174	331	5
i	4	181	0.8757	1.5023	...	...	172	316	4
j	12	185	0.8755	1.4998	...	...	165	280	2
k	10	198	0.8838	1.5050	...	...	167	297	4
l	12	209	0.9141	1.5257	...	...	187	376	3
m	17	223	0.9027	1.5134	...	...	166	287	3
n	2	233	0.9529	1.5556	...	...	217	506	5
o	19	244	0.9384	1.5401	...	...	193	400	3
p	1	254	0.9442	1.5460	...	...	197	432	7
q	6	257	0.9648	1.5675	...	...	232	564	4
r	11	266	0.9550	1.5535	...	...	208	457	2
s	7	274	0.9538	1.5527	...	...	207	448	1
t	4	280	0.9590	1.5585	...	...	215	483	2
u	11	288	0.9583	1.5546	...	...	206	443	1
v	14	300	0.9557	1.5497	...	...	197	399	− 1
w	8	311	0.9617	1.5523	...	...	198	396	− 3
x	7	318	0.9648	1.5527	...	...	195	385	− 2
y	21	332	0.9706	1.5561	...	...	197	389	− 3
z	18	352	0.9778	1.5619	...	...	204	412	− 5
6aa	80	146	0.8660	1.4943	...	...	176	328	3
ba	90	232	0.9231	1.5330	...	...	193	394	2
23a catalytic fraction	15	136	0.8638	1.4962	...	...	180	343	2
b	13	143	0.8739	1.5019	...	...	179	353	6
c	1	163	0.8650	1.4973	...	...	174	322	3
d	1	168	0.8735	1.5050	...	...	177	361	10
e	1	172	0.8774	1.5054	...	...	178	349	6
f	2	183	0.8700	1.5003	...	...	170	315	5
7a fuel	110	198	0.9115	1.5292	...	...	200	431	4
8a	150	185	0.8873	1.5088	...	...	177	333	3
9a	80	203	0.8876	1.5064	...	...	168	292	2
24a thermal fuel oil	...	...	0.9617	1.5535	28.7	3.97	200	381	−9
b	...	...	1.0212	1.6069	77.7	5.71	262	669	0
c	...	...	1.0471	1.6318	118.9	6.42	296	813	1
d	...	...	1.0617	1.6452	103.6	5.85	310	891	7
20a straight run oil	...	...	1.0055	1.5727	235.0	8.9	195	387	− 2
b	...	...	0.9968	1.5606	869.1	14.9	172	298	− 1
c	...	...	0.9661	1.5354	482.8	13.6	144	169	− 4
							Average deviation		3
							Deviation of average		2
							Maximum deviation		10

Table IV. Agreement of Petroleum Fractions, Pulfrich Refractometer Data

	Magnitude Boiling Range, °C.	50% B.P., °C.	d_4^{20}	n_D^{20}	Viscosity, Centistokes 100° F.	Viscosity, Centistokes 210° F.	Av. Mol. Wt.	δ	$10^4 \times n - n_{calcd.}$	C	$\delta_{calcd.}$	$\delta_{calcd.} - \delta$
Straight run gasoline	125	120	0.7521	1.4199	...	...	112	107	26	2	107	0
Catalytic gasoline	130	108	0.7555	1.4253	...	...	104	122	79	2	120	−2
Thermal gasoline	180	149	0.7910	1.4429	...	...	126	124	80	3	121	−3
a Catalytic gasoline cut	30	79	0.7183	1.4013	...	...	92	103	15	2	104	1
b Catalytic gasoline cut	25	107	0.7752	1.4352	...	....	101	128	104	2	126	−2
c Catalytic gasoline cut	25	136	0.8159	1.4628	...	...	113	148	192	2	147	−1
d Catalytic gasoline cut	25	163	0.8388	1.4775	...	...	127	154	222	3	156	2
a Straight-run fuel cut	75	132	0.7603	1.4242	...	...	118	107	28	3	108	1
b Straight-run fuel cut	110	226	0.8143	1.4550	...	...	178	116	58	4	117	1
Fuel	140	216	0.8506	1.4785	...	...	164	140	151	4	139	−1
Fuel	150	174	0.7718	1.4318	...	...	144	108	28	3	108	0
Fuel	90	198	0.8364	1.4585	...	...	152	106	19	4	107	1
Thermal gas oil	130	267	0.8549	1.4780	...	...	205	128	103	5	129	1
Thermal gas oil	120	236	0.9014	1.5079	...	...	170	157	229	4	158	1
Catalytic gas oil	130	266	0.8694	1.4907	...	...	201	146	171	5	145	−1
Catalytic gas oil	140	267	0.8613	1.4811	...	...	202	130	109	5	130	0
Straight-run gas oil	140	263	0.8323	1.4652	...	...	205	120	70	5	120	0
Straight-run gas oil	110	283	0.8818	1.4836	...	...	215	114	42	5	114	0
Lube oil	...	...	0.9173	1.5060	22.1	3.60	300	128	92	7	128	0
Lube oil	...	...	0.9126	1.5028	22.4	3.69	308	125	78	7	124	−1
Lube oil	...	...	0.9244	1.5098	46.3	5.34	332	130	93	7	128	−2
Lube oil	...	...	0.9253	1.5105	61.6	6.17	347	129	93	7	128	−1
Lube oil	...	...	0.9325	1.5152	104.7	8.0	362	133	107	8	133	0
Lube oil	...	...	0.9311	1.5144	150.7	9.9	387	131	101	8	131	0
Lube oil	...	...	0.9390	1.5186	470.8	17.5	426	132	104	8	132	0
										Average deviation		1
										Deviation of average		0
										Maximum deviation		−3

countered in the petroleum industry. To obtain a more accurate correction term, it would be necessary to know the magnitude of difference in the physical properties and the relative concentrations of the components. This information is not generally available and would be difficult to obtain.

ble V. Agreement of Petroleum Fractions, Abbe Refractometer Data

(Averaged by Group)

	No. of Fractions	Mol. Wt.	δ	$10^4 \times n - n_{calcd.}$	C	$\delta_{calcd.}$	$\delta_{calcd.} - \delta$
troleum fractions							
Kreulen (*5*), gas oils	11	219	130	113	5	131	1
Fenske (*1*), lube oils							
Penna. 180 neutral	22	393	115	53	8	119	4
Rodessa neutral	16	396	107	49	8	118	11
Midcontinent neutral 1	18	380	126	85	8	127	1
Midcontinent neutral 2	18	366	126	89	8	128	2
California neutral[a]	17	303	137	136	7	139	2
Gulf Coast neutral	18	345	130	101	7	130	0
				Average deviation per fraction			4
				Deviation of average per fraction			+3
tracts from lube oils							
Fenske (*1*)							
Bosco neutral extracts	12		135	145		134	−1
Bosco neutral extracts	11		96	−10		98	2
Bosco neutral, H_2SO_4, $AlCl_3$, and clay-treated fractions	9		106	49		110	4
Midcontinent 2 extracts	4		137	166		139	2
Midcontinent 2 extracts	5		92	12		101	9
Mair and Schicktanz (*13*)							
Midcontinent extracts	7		114	48		110	−4
Midcontinent extracts	17		99	−13		98	−1
Mair and Willingham (*14*)							
Midcontinent extracts	52		175	302		172	−3
Hydrogenated extracts	15		100	−12		98	−2
				Average deviation per fraction			4
				Deviation of average per fraction			−1

[a] Two fractions obviously in error omitted from tabulation.

ACCURACY

Table II presents the data on 362 pure hydrocarbons of all types and degrees of unsaturation. The average deviation (without regard to sign) of the calculated specific dispersion is 7 units. Nine compounds have been omitted from this average because of their wide deviation from the average line, Figure 1. These compounds fall into two classes whose specific dispersion is extremely high, the anthracenes and aromatics with conjugated diolefin side chain. The calculated specific dispersions of these nine compounds are lower than the experimental values. The authors intend to make a further investigation of this deviation.

The averages for all other classes, including acetylenes and conjugated diolefins, scatter on both sides of the line as is demonstrated in Figure 1. The groups showing the larger numerical deviations are usually of higher specific dispersion, so that deviations considered on a percentage basis are about the same. Triolefins and other types which polymerize readily, and which are difficult to synthesize and analyze, show the poorest agreement with the equation.

These pure hydrocarbon data are obtained from the sources listed previously (*12*) and from Thorpe and Larsen (*17*).

Table III presents data obtained in this laboratory on aromatic extracts from petroleum. These fractions include straight-run, thermal, and catalytic materials of narrow and wide boiling range, from the gasoline to lube oil boiling ranges. Specific dispersions were obtained on the Pulfrich refractometer and are accurate within ±0.5 unit. The average deviation for 42 fractions is 3 units and the maximum deviation is 10 units.

Table IV presents data obtained in this laboratory on various petroleum fractions containing olefins, aromatics, paraffins, and naphthenes. For these materials it was necessary to calculate specific dispersions using the additive corrections because these fractions are mixtures of different hydrocarbon types. Average deviation for 25 fractions is 1 unit, maximum deviation is −3 units.

Table V shows literature data for 252 petroleum fractions on which dispersions have been determined with the Abbe-type refractometer. The average deviation is 4 units, which is comparable with the experimental accuracy of the data.

Nonhydrocarbons will, of course, affect the calculation, but are probably present in concentrations too small to be significant.

The data in Tables III and IV demonstrate that the specific dispersion of petroleum fractions may be calculated with an ac-

curacy of ±3 units. This is comparable with experimental determination on the Abbe-type instrument which is, according to von Fuchs (*2*), accurate within 3 dispersion units.

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Determination of Copper in Cast Iron and Steels with Quinaldic Acid

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The method of Zan'ko and Butenko for the determination of copper in steel by precipitating with quinaldic acid has been investigated and extensively modified. Satisfactory results are obtained only after a reprecipitation of the copper quinaldate. The speed of the method may be increased by making a preliminary separation of copper by internal electrolysis, using an aluminum anode. Spectrographic studies showed precipitates to be of sufficient purity for reliable results. The method, as applied to Bureau of Standards steels, gave an accuracy of about 2 to 3% on samples containing 0.1% or more of copper, but less at lower percentages of copper. The precision is about the same as the accuracy.

METHODS available for determining copper in cast iron and steel include hydrogen sulfide precipitation (*13*), direct electrolysis (*5*, *10*, *12*, *15*), polarography (*9*), spectrographic methods (*18*), and precipitation with organic reagents. Among the reagents whose use as precipitants for copper has been recommended are salicylaldoxime (*7*, *16*), benzotriazole (*2*), α-benzoinoxime (*8*, *14*, *17*), 5,7-dibromo-8-hydroxyquinoline (*19*), and quinaldic acid (*20*).

The method described in this paper employs quinaldic acid as the precipitant for copper. The method has been studied in some detail in order to obtain a clear picture of the conditions necessary for precipitation, the sources of interference, and, finally, its ultimate accuracy. (Studies were also made using 5,7-dibromo-8-hydroxyquinoline. The extreme insolubility of this reagent in water caused its precipitation, in many cases, along with the copper salt, and its further use was not investigated.)

Quinaldic acid was chosen as the precipitant because of its solubility in water and stability in solution, the definite composition of its salts and their easy filtration, and because information on the behavior of other quinaldates was available (*3*). The method of investigation has been to test the precipitation of copper under conditions similar to those encountered in steel analysis, and then to analyze Bureau of Standards steels.

MATERIALS AND APPARATUS

Quinaldic acid was prepared by the method of Hammick (*6*) from quinaldine, Eastman No. 216. The product was recrystallized from glacial acetic acid and melted at 156–156.5° (uncorrected). Hammick gives 156° (uncorrected) as the melting point. A 1% solution of the acid in water neutralized to pH 7 with sodium hydroxide was used as a reagent.

Standard copper solutions were prepared by dissolving accurately weighed samples of freshly cleaned pure electrolytic copper foil in dilute nitric acid and diluting to volume.

Ferric salts, for use in making synthetic solutions, were made by dissolving Baker's primary standard iron wire in the appropriate acids. Baker's C.P. iron powder, by hydrogen, was also used and has the advantage of dissolving more rapidly. However, it contains sufficient insoluble material to require filtration of its solutions before use.

Tartaric acid was Merck's reagent grade, fine crystals. Oxalic acid was U.S.P. grade. Hydroxylamine hydrochloride was the Eastman material and was generally dissolved in a small amount of water immediately before use.

All other reagents and chemicals were of the highest purity obtainable. Normax volumetric ware was used throughout. The weighings were performed in a new Seko analytical balance with a sensitivity of 0.2 mg. per scale division and a reliability determined as ±0.03 mg. by the method of Benedetti-Pichler (*1*). A Beckman pH meter with glass electrode assembly, frequently tested against carefully prepared standard buffers, was used for the pH measurements.

EXPERIMENTAL

A. Double Quinaldic Acid Precipitation. The precipitation of metals from solution with quinaldic acid has been investigated theoretically by Flagg and McClure (*3*). The following procedure was followed to check the reliability of the determination of a small amount of copper from a pure standard solution.

Sufficient standard copper solution to contain 1.008 mg. of copper was pipetted into a 250-ml. beaker and 5 ml. of glacial acetic acid were added. The solution was diluted to 95 ml. and the pH adjusted to 5.5 with dilute sodium hydroxide. The mixture was then heated to 70° to 80° C. and 5 ml. of the 1% quinaldic acid (50 mg.) solution were added slowly. The precipitate was digested 15 or 20 minutes on a steam bath and allowed to cool to room temperature before filtering. After the precipitate was collected on a weighed Pyrex 30F filtering crucible and washed several times with water, it was brought to constant weight at 115–120° C. Two such determinations gave an average value of 1.012 ± 0.009 mg.

The procedure was then repeated, substituting oxalic or tartaric acid for acetic acid. No precipitate appeared in any of several trials using 2 grams of oxalic acid at a pH of 5.5. Two runs in which 2 grams of tartaric acid and a pH of 5.5 were used gave an average value of 1.013 ± 0.003 mg. for the weight of copper. Evidently the complex formed by cupric ion with oxalate is too stable to permit the precipitation of copper quinaldate at this pH.

The high pH value was used because the stability of the complexes formed with oxalate and tartrate increases with increasing H, so that any interference of these ions with the precipitation f the copper will be magnified. In practice, quantitative precipitation can be obtained under these conditions with a pH as ow as 2.6. Obviously, copper can be quantitatively precipitated as the quinaldate in the presence of a large excess of tartrate.

When test analyses were begun on synthetic solutions containing 1 gram of iron (III) and 6 grams of tartaric acid, a difficulty was encountered in the precipitation of large quantities of basic ferric tartrates on neutralization. Experimentation showed that the basic salts do not appear if the iron was originally present as the nitrate or sulfate, but appear only when much of the iron was there as the chloride. Hence, thereafter, the iron for the synthetic mixture was dissolved in a mixture of nitric and sulfuric acids.

The procedure used for the analysis of synthetic mixtures was as follows:

To the pipetted volume of the standard copper solution were added the solution of 1 gram of iron as ferric sulfate and 6 grams of tartaric acid. The solution was brought to the desired pH with concentrated ammonium hydroxide and an excess of quinaldic acid was added after heating to 70° to 80° C. Filtration, etc., were as in the determinations described above. In every case the precipitates were contaminated with ferric quinaldate, as evidenced by their color. Several different wash liquids were tried, but none was successful in removing the contamination.

Results obtained are given in Table I. The amount of iron coprecipitated is seen to vary irregularly with the pH. In runs similar to those reported in Table I the amount of iron in the precipitate was estimated and up to 0.03 mg. was found. Obviously the single precipitation does not give a satisfactory separation of copper from such a large quantity of iron. A double precipitation procedure was then tried on standard steel samples obtained from the Bureau of Standards.

Recommended Procedure. A factor weight (1.493 grams) sample of the material is dissolved in 15 to 20 ml. of aqua regia. When the material has dissolved completely, 10 ml. of dilute sulfuric acid (1 to 1) are added and the solution is evaporated to dense white fumes of sulfur trioxide. The residue is taken up in 50 ml. of water and as much dilute sulfuric acid (1 to 4) as necessary to effect solution of the salts. Next the silica and other insoluble material are filtered off on Whatman No. 40 filter paper. After the silica is washed once with water, it is washed with 25 ml. of a solution containing 10 grams of ammonium tartrate, and then once more with water. Concentrated ammonium hydroxide is then added to the combined filtrate and washings until it turns a deep cherry red color. Upon cooling the pH is adjusted to 3.0 ± 0.2 with dilute ammonium hydroxide (1 to 3) or dilute sulfuric acid (1 to 4). The solution is then heated to 70° to 80° C. and 2 ml. of 1% quinaldic acid solution are added per milligram of copper present. Solution and precipitate are digested on the steam bath for half an hour and then allowed to cool to room temperature. The precipitate is collected on the filtering crucible and washed once or twice with cold water.

The filtering crucible is placed in an apparatus designed for quantitative recovery of filtrate and the precipitate is dissolved in a few milliliters of hot dilute hydrochloric acid (1 to 1). The crucible is rinsed with several small portions of hot acid and finally with water. To the filtrate 2 grams of tartaric acid and 2 ml. of the quinaldic acid solution are added and the pH is adjusted to about 3 with dilute ammonium hydroxide (1 to 4). The precipitate is digested at 70° to 80° C., cooled and filtered as before, this time on a weighed glass crucible, and washed with cold water. The crucible is dried at 115° to 120° C. for one hour and weighed. The weight of the precipitate in milligrams divided by 100 gives the percentage of copper in the sample.

Table I. Determination of Copper by Precipitation with Quinaldic Acid from Synthetic Mixtures

Cu Taken	pH	Quinaldic Acid Used	Weight of Precipitate	
			Theoretical	Actual
Mg.		*Mg.*	*Mg.*	*Mg.*
1.01	3.4	50	6.77	10.18
1.01	5.8	50	6.77	7.64
5.04	2.4	100	32.75	36.28
5.04	3.7	100	32.75	37.99
5.04	4.5	100	32.75	36.12

Table II. Determination of Copper in Standard Steels by Double Quinaldic Acid Precipitation

B. of S. No.	Type	First Precipitation	Second Precipitation	Averages	B. of S. Certificate Value
		%	%	%	%
111	Ni-Mo	0.151	0.115		
		0.146	0.117		
		0.136	...		
		0.127	...		
		...	0.121		
				0.118	0.122
106	Cr-Mo-Al	0.162	...		
		0.166	0.140		
		0.156	0.139		
				0.140	0.142
101	18–8	0.075	0.066		
		...	0.055		
				0.061	0.055
5h	Cast Fe	1.57	1.46		
		1.45	1.40		
		1.61	1.46		
		1.53	1.47	1.45	1.45
11e	Plain, low C	0.114	0.103		
		0.117	0.103		
				1.103	0.105
125	High Si	0.081	0.067		
		0.066	0.055		
		0.070	0.052		
				0.058	0.066
30c	Cr-V	0.092	0.092		
		0.110	0.106		
				0.099	0.099
55a	Ingot iron	0.043	0.043		
		0.046	0.043		
				0.043	0.046
8d	Bessemer	0.011	0.011		
		0.015	0.010		
				0.011	0.013

The values given in Table II were obtained by this method. In most cases the copper quinaldate from the first precipitation was dried and weighed and that value is given also. Perchloric acid digestion was tried because of the difficulty encountered in dissolving the basic salts formed in the sulfuric acid evaporation, particularly since hydrochloric acid cannot be used to aid in redissolving them. However, solutions prepared in this manner tend to give precipitates of basic tartrates similar to those that form when chloride is used. It was found, in general, that it is not necessary to redissolve the basic salts formed in the evaporation quantitatively before filtering in order to obtain good results. The pH meter need not be used in these precipitations. The precipitations may be carried out satisfactorily at a somewhat higher pH, using nitrazine paper for the first neutralization and methyl orange for the second. The Filtrator, obtainable from the Fisher Scientific Company, Pittsburgh, Pa., was found to be most convenient in obtaining quantitative recovery of the filtrate after dissolving the impure copper quinaldate from the first precipitation.

The silica precipitates, reprecipitation filtrates, and copper quinaldate precipitates from several of the analyses reported in Table II were reserved for qualitative spectrographic analysis. The spectrum plates were taken with a Bausch & Lomb large Littrow spectrograph and analyzed in a roughly quantitative manner by the projection method. The results showed definitely that the copper quinaldate precipitates were not contaminated with weighable quantities of any elements. Further, the quantities of copper lost to the silica precipitates and the reprecipitation filtrates are also negligible. Hence it is likely that the favorable results reported in Table II are not the result of compensating errors. The copper quinaldate precipitates were generally free from iron; occasional traces of nickel, zinc, silver, tin, titanium, lead, chromium, cobalt, and manganese were found. Notably absent were the elements silicon, tungsten, vanadium, and molybdenum.

B. Preliminary Separation by Internal Electrolysis. The separation of copper from iron by internal electrolysis using

Table III. Determination of Copper by Internal Electrolysis from Ferrous Chloride Solution

Cu Taken	Time of Electrolysis	Cu Found
Mg.	*Min.*	*Mg.*
5.04	45	4.98
5.01	75	5.04
5.01	30	5.00
1.00	75	1.04
1.00	30	1.04

Table IV. Determination of Copper in Steels by Quinaldic Acid Precipitation after Separation by Internal Electrolysis

B. of S. No.	Type	B. of S. Certificate Value	% Cu Found
111	Ni-Mo	0.122	0.120
11e	Plain, low C	0.105	0.102
125	High Si	0.066	0.061
50a	Cr-V-W	0.047	0.043
55a	Ingot iron	0.046	0.048
101	18–8	0.055	0.035

an aluminum anode has been described by Lurie and Ginsburg (*11*). An extension of their work by Flagg and Underwood (*4*) indicated that the separation of milligram quantities of copper from 1 gram of iron was at least feasible. The technique is simpler and in general more rapid than external electrolytic methods and appeared to offer a method applicable to steel analysis.

The apparatus used was similar to that of Lurie and Ginsburg (*11*). The cathode was an ordinary platinum gauze electrode, the anode a 23-cm. length of Baker's 99.99% aluminum wire bent into a spiral or simple loop. The electrolysis and preliminary operations were carried out in an electrolysis cell made by cutting a 38 × 300 mm. Pyrex test tube to a length of 13 cm. Test analyses were run from a solution containing 1 gram of iron as ferrous chloride. To the iron solution in the electrolysis vessel were added the pipetted volume of standard copper solution, 1 ml. of concentrated hydrochloric acid, and 1 ml. of concentrated nitric acid. The cell was then immersed in a beaker of water held at 70° to 80° C. by a steam bath and the electrolysis continued for the time indicated. At the end of the run the electrodes were removed with a stream of water playing on the cathode, rinsed in distilled water and acetone, and dried and weighed in the usual manner. Results obtained are shown in Table III.

Analyses of Bureau of Standards nickel-molybdenum steel, No. 111, were then attempted. A 1-gram sample was weighed in the electrolysis vessel and dissolved in aqua regia. Three milliliters of concentrated sulfuric acid were added and the solution was evaporated to fumes. The residue was taken up with about 10 ml. of water, and boiled, and 1.2 grams of hydroxylamine hydrochloride in 10 ml. of water were added to reduce ferric ion to ferrous. Two milliliters of dilute hydrochloric acid (1 to 1) and 2 ml. of dilute nitric acid (1 to 3) were added and the electrolysis was carried out as above.

Six such analyses gave a value of 0.157 ± 0.021% copper as compared with the standard analysis of 0.122%. The poor precision is explained by the possibility of a varying amount of insoluble silica and graphite being occluded in the deposit. A similar effect was noted by MacNeven and Bournique (*12*) in external microelectrolysis and was avoided by filtering before electrolysis. The high results are due to this occlusion and to the presence of small amounts of other metals which can be deposited under these conditions. Their presence was confirmed by spectrographic analysis of three of the deposits, all of which showed fairly strong lines of silver, bismuth, and tin. The combined percentages of these elements would not have to exceed 0.02% to account for the above error. However, it was found that this interference can be eliminated if the deposit be stripped and the copper determined by quinaldic acid precipitation.

Recommended Procedure. The deposits are stripped, after dissolving and electrolyzing the sample as described above, with concentrated nitric acid in an apparatus similar to that described by Silverman, Goodman, and Walter (*15*). The solutions are then run into 150-ml. beakers and 5 grams of ammonium acetate are added. The pH is adjusted to 3 to 3.5, but methyl orange may be used with equally satisfactory results. The copper is then determined by precipitating with 50 mg. of quinaldic acid; filtration, washing, and drying of the precipitates are as described in Section A, recommended procedure. Results obtained on several Bureau of Standards steel samples are given in Table IV.

The values obtained are satisfactory in most cases. The 18–8 steel, No. 101, at the point of electrolysis had suspended in the solution large amounts of basic chromic sulfate, which may have prevented complete deposition of the copper. The solution was usually sufficiently acid after the evaporation, so that additional acid was unnecessary. In this case the chloride and nitrate ions necessary to the smooth performance of the electrolysis were added as the sodium salts instead. In the case of readily soluble materials, such as the ingot iron, No. 55a, the evaporation can be omitted entirely, as the acid concentration when solution is complete will be just about that required for smooth electrolysis.

Spectrographic analysis showed that the precipitate is as a rule somewhat less pure than that obtained by a double quinaldic acid precipitation but that the quantities of the elements present are still negligible.

ADVANTAGES AND DISADVANTAGES

Advantages. Use of simple and readily available apparatus, an advantage over conventional electrolytic methods; precipitate of definite composition; stable reagent readily soluble in water.

The average cost of materials per duplicate determination is about 3 cents. The reagent can be recovered by dissolving the precipitates with hydrochloric acid, precipitating the copper with hydrogen sulfide, filtering, and recovering the quinaldic acid from the filtrate by evaporation.

Disadvantages. Necessity for reprecipitation, making for a somewhat slower process.

The method is no more accurate than methods previously in general use. In many steels, the accuracy is limited by the small weight of precipitate obtained.

Complete removal of chloride ion is absolutely necessary in the quinaldic acid procedure in order to avoid precipitation of basic ferric tartrate.

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Determination of Rubber Hydrocarbon by a Gravimetric Rubber Bromide Method

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A direct method is given for determining rubber hydrocarbon in extracts from plant tissues, and in rubber crudes, latices, and liquors. The method is based upon the formation of an alcohol-insoluble rubber bromide whose composition varies slightly, depending on the source of the rubber. Gravimetric factors are established for converting weights of rubber bromide to weight of rubber, when the source is guayule, Cryptostegia, or kok-saghyz. Data are presented showing that reasonable variations in time and temperature of bromination and changes in concentration of rubber and brominating solution, within rather wide limits, do not appreciably alter the accuracy of the method.

DIRECT determination of rubber hydrocarbon in crude rubbers and in latices has been generally considered so inaccurate and unreliable that determination of rubber hydrocarbon by difference has been a common practice. The difference method usually suffices in the analysis of products derived from Hevea rubber, in which the nonrubber constituents have been well characterized or occur in low concentration. In the analysis of natural rubber products obtained from sources other than Hevea, such as guayule, kok-saghyz, and Cryptostegia, the situation is different. The chemical and physical properties of the nonrubber constituents of such products are largely unknown, and in many cases the nonrubber contaminants are present in excessive amounts, sometimes more than 50% of the total. Since the rubber hydrocarbon analysis usually affords the most reliable means for estimating the rubber content of plants and for following the concentration and purification of rubber by mechanical and chemical processes, it appeared desirable to devise or adapt a method which would permit direct determination of this constituent in the presence of relatively large amounts of natural and added contaminants.

Among the methods for determination of rubber in the literature, those which have received the largest amount of study are the gravimetric (*1*, *3*, *5*, *10*, *12*) and volumetric (*7*, *11*, *13*) methods based on the formation of the bromine derivative of rubber. Other methods are the empirical chromic acid oxidation (*4*), the direct alcohol precipitation (*6*), and the nitrosite procedures (*9*,). The gravimetric bromide method showed the greatest promise of adaptability to the determination of rubber hydrocarbon in a wide variety of rubber-containing products. Both the volumetric bromide method and the chromic acid oxidation method are subject to error when the rubber contains material other than rubber hydrocarbon, since any nonrubber compounds capable of reacting with bromine interfere in the volumetric bromide method and any constituents yielding volatile acids on digestion with chromic acid tend to give high results if the chromic acid oxidation method is employed. Estimation of rubber by direct weighing of the hydrocarbon precipitated with alcohol is not applicable to the determination of rubber in impure or dilute solutions. The nitrosite method was not investigated, since it has never met with favor because of the uncertain composition of the reaction products. The gravimetric bromide method was therefore selected for further investigation as a possible and practical means for the direct estimation of rubber hydrocarbon.

The bromine derivative of rubber or the so-called "tetrabromide" was first prepared by Gladstone and Hibbert (*8*), who isolated the compound by evaporating the solvent from a chloroform-rubber solution that had been treated with bromine. Weber (*14*) found that the compound could be more conveniently isolated by precipitation with alcohol. Analysis of his preparation showed a composition in essential agreement with the formula $C_{10}H_{16}Br_4$. The methods devised for determination of rubber hydrocarbon based on the formation of the bromide vary in the choice of (*a*) rubber solvent (carbon tetrachloride, chloroform, or benzene), (*b*) brominating conditions such as time, temperature, and composition and concentration of the reagent, and (*c*) bromide precipitant (alcohol, acetone, or petroleum ether). In several of the methods the bromine in the isolated bromide precipitate is determined and the amount of rubber hydrocarbon calculated by using the ratio of 1 isoprene unit (C_5H_8) to 2 bromine.

Several early investigators recognized that this theoretical ratio was not obtained in practice, since formation of the bromide was generally accompanied by liberation of hydrogen bromide, indicating that substitution as well as addition of bromine took place. Hinrichsen and Kindscher (*10*) observed no liberation of hydrogen bromide if the bromination was carried out at 0° C., and proposed a method based on this finding. The volumetric bromide method of Lewis and McAdams (*11*) attempted to evaluate the amount of substitution by titration of the liberated hydrogen bromide and corrected the results accordingly. Bloomfield (*2*) has recently reported that when bromination of rubber is carried out at temperatures below 0° C. in a medium containing a trace of alcohol, substitution of bromine is minimized. A simple adaptation of the bromide method was made by Edison (*5*) and his associates for use in their survey of native American plants as potential sources of natural rubber. In this method, a benzene or carbon tetrachloride solution of rubber is brominated for one hour, the bromide is precipitated with alcohol, washed, dried, and weighed, and the rubber hydrocarbon content is calculated by multiplying the weight of recovered bromide by an empirical factor, 0.285, instead of the theoretical factor, 0.299.

Since conversion of weight of rubber bromide to weight of rubber is based on an empirical factor, it was necessary to establish this factor for rubber from different sources. Effects of variations in conditions were studied to determine the limits within which it is possible to use the established factor.

EXPERIMENTAL

Samples of rubber bromide for ultimate analysis were prepared by the bromination of benzene solutions of rubber derived from various Cryptostegia, guayule, and kok-saghyz products. Benzene solutions of the rubber of each plant species were obtained as benzene extracts of the acetone-extracted plant tissues, solutions of the resin-containing crude rubber, milled, or extracted from the plants, or extracts of latices or plant liquors prepared from the plants.

Accurately measured volumes of such rubber solutions containing between 2 and 2.5 mg. of rubber per ml. were brominated at room temperature by adding 1 ml. of the brominating solution (see reagents) for every 10 ml. of rubber solution. Bromination was stopped and the bromide precipitated at the end of an hour by the addition of 30 ml. of absolute alcohol for every 10 ml. of the brominated mixture. The bromide was allowed to settle for 2 hours and was then filtered, washed with absolute alcohol, and dried to constant weight at 50° C. in vacuo. The dried precipitates were analyzed for carbon and hydrogen and in some cases for bromine. When the bromide was separated by filtration from the mother liquor immediately after precipitation, the weight and composition of the recovered bromide were the same as those of the bromide separated after standing for 2 hours.

All analyses of carbon, hydrogen, and bromine were made by common microprocedures. Many analyses were made of bromides prepared from resin-free solvent-extracted rubber, crude rubber, and rubber latex obtained from each of the three plant

Table I. Composition of Rubber Bromides[a] from Three Plant Sources

Plant Source	Product from Which Prepared	Bromine, %	Carbon, %	Hydrogen, %	Sum of C + H, %	Total, %
Guayule	Latex dispersion	...	25.9	3.4	29.3	..
	Crude rubber	...	25.3	3.5	28.8	..
	Acetone-extracted plant tissue	69.8	25.9	3.5	29.4	99.2
Av.			25.7	3.5	29.2	
Kok-saghyz	Crude rubber	69.9	25.7	3.2	28.9	98.8
	Acetone-extracted plant tissue	70.4	25.9	3.3	29.2	99.6
Av.			25.8	3.2	29.0	
Cryptostegia	Crude rubber	69.2	27.0	3.4	30.4	99.6
	Acetone-extracted plant tissue	69.9	26.9	3.4	30.3	100.2
Av.			26.9	3.4	30.3	
Theoretical composition of $C_5H_8Br_2$		70.12	26.34	3.54	29.88	

[a] Each value is average of values obtained in three to eight determinations.

Table II. Effect of Volume Ratio of Brominating Reagent to Rubber-Benzene Solution on Weight of Rubber Precipitated

Br Solution per 10 Ml. of Rubber-Benzene Solution	Kok-saghyz: Rubber bromide from 20 ml. of rubber-benzene	Kok-saghyz: Wt. of rubber calculated[a]	Guayule: Rubber bromide from 20 ml. of rubber-benzene	Guayule: Wt. of rubber calculated[b]
Ml.	*Grams*	*Grams*	*Grams*	*Grams*
1	0.1133	0.0329	0.1327	0.0387
2	0.1124	0.0326	0.1345	0.0393
3	0.1135	0.0329	0.1348	0.0394
4	0.1145	0.0332	0.1355	0.0396
5	0.1159	0.0336	0.1350	0.0394
6	0.1143	0.0331	0.1363	0.0398

[a] Gravimetric factor 0.290.
[b] Gravimetric factor 0.292.

sources. The average values in Table I were obtained by three to eight determinations of bromides from each source.

The sums of the averages of the carbon and hydrogen content give the factors for converting weights of rubber bromides to weights of rubber. The factors obtained are as follows:

Guayule	0.292
Kok-saghyz	0.290
Cryptostegia	0.303

A mean deviation of ±0.003 for each of the three factors was obtained when the carbon and hydrogen data for all the samples were analyzed. In general usage it is not necessary to employ the two factors, 0.292 and 0.290, for guayule and kok-saghyz, respectively, but rather to use the average factor, 0.291, whose mean deviation is within the limits of experimental error.

As an additional test of the variation of the gravimetric factor for rubber in rubber bromide, many of the benzene-rubber extracts obtained in the course of plant analyses were analyzed for rubber both by the bromination procedure and by weighing the rubber film obtained by evaporation. Ultimate analyses showed that the films were 98 to 99% pure. The gravimetric factors were determined by dividing the weight of the rubber film by the weight of the rubber bromide. The average of more than one hundred such determinations yielded the following factors: guayule, 0.292 ± 0.003; kok-saghyz, 0.290 ± 0.004; and Cryptostegia, 0.302 ± 0.003. All are in close agreement with the values obtained by the ultimate analysis of the bromides (Table I). Preliminary experiments have indicated that this method is applicable to Hevea and that the factor 0.291 gives satisfactory results. In addition, test analyses on a sample of GR-S synthetic rubber indicated that reliable results are obtainable using an experimentally found factor of 0.342.

To evaluate variations in time, temperature, rubber concentration, and bromine concentration which would not significantly alter the factor, the weights of bromide recovered from aliquots of rubber-benzene solutions brominated under various conditions were compared. Table II shows the effect of varying the volume ratio of the brominating solution to the rubber-benzene solution.

Although there is a slight increase in the weight of rubber bromide formed with increased amounts of the brominating solution, the weights of rubber, calculated by the factor previously established by ultimate analyses, are fairly constant. Since the variation caused by increasing amounts of brominating reagent is small, the procedure adopted has been to use 1 ml. of reagent for every 10 ml. of rubber-benzene solution, as the gravimetric factors have been established on the basis of this ratio. To change the ratio would necessitate establishing new factors which would be even further from the theoretical value of 0.299.

To show the effect of varying rubber concentration on the amounts of rubber calculated from the bromide with the established factor, a series of 10-ml. aliquots of a rubber-benzene solution was diluted with different amounts of benzene. These were brominated by the addition of 1 ml. of brominating solution for every 10 ml. of the rubber-benzene solutions. Table II shows the results.

It is evident that rubber-benzene solutions containing 0.5 to 3.0 mg. of rubber per ml. of solution can be analyzed by this procedure, with a recovery of 98 to 100%. For solutions containing much more than 3.0 mg. of rubber per ml., low values were obtained.

Aliquots of benzene solutions of rubber from three plant sources were brominated at room temperature (25° ± 2.5° C.) and in a refrigerator at 4° C. for periods ranging from 1 to 24 hours. One milliliter of brominating reagent was used for every 10 ml. of rubber-benzene solution. Table IV shows the results.

The data indicate that reasonable variations in time and temperature of bromination from the one hour and room temperature of the described procedure cause insignificant changes in the amounts of rubber calculated from the bromides. However, prolonged bromination at room temperature results in a gradual increase in the weight of the precipitate; this is presumably due to substitution of bromine. At 4° C. the same phenomenon occurs, but, as would be expected, the rate is slower. When brominations were made at −4° C. in carbon tetrachloride solution containing a trace of alcohol, the gravimetric factors calculated for rubber in rubber bromide formed approached the theoretical value. The proposed method of brominating at room temperature is preferred, however, because of its ease of operation, its adaptability, and the accuracy obtainable.

Results of the carbon and hydrogen analyses of the bromides indicate that the gravimetric factors for converting weights of bromide to weights of rubber vary from one plant source to another. The cause of these variations has not been determined; they may be due to the inherent nature of the rubbers. That the bromide method accounts for all the rubber in a solution is

Table III. Analysis of Solutions of Different Rubber Concentrations

Guayule: Mg. of Rubber per 10 Ml. of Rubber-Benzene Solution, Added	Guayule: Found[a]	Guayule: % Recovery	Kok-saghyz: Mg. of Rubber per 10 Ml. of Rubber-Benzene Solution, Added	Kok-saghyz: Found[a]	Kok-saghyz: % Recovery	Cryptostegia: Mg. of Rubber per 10 Ml. of Rubber-Benzene Solution, Added	Cryptostegia: Found[a]	Cryptostegia: % Recovery
6.6	6.6	100	5.6	5.6	100	5.4	5.4	100
7.9	7.8	99	6.6	6.6	100	6.5	6.5	100
9.9	9.7	98	8.2	8.2	100	8.2	8.1	99
13.2	13.1	99	10.9	10.7	98	10.9	10.8	99
19.8	19.6	99	16.4	16.4	100	16.3	16.1	99
29.7	29.5	99	24.6	24.5	100	24.5	23.9	98
39.5	37.4	95	32.8	31.9	97	32.6	31.5	97

[a] Gravimetric factors: guayule, 0.292, kok-saghyz, 0.290; Cryptostegia, 0.303. 1 ml. of brominating reagent added for each 10 ml. of rubber-benzene solution.

able IV. Effect of Time and Temperature of Bromination on Weight of Rubber Found

Time of Bromination	Weight of Rubber Calculated[a] from Bromide Precipitated from 20 Ml. of Solution					
	Cryptostegia		Kok-saghyz		Guayule	
	Room temp.	4° C.	Room temp.	4° C.	Room temp.	4° C.
Hours	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*
1	0.0162	0.0162	0.0276	0.0271	0.0405	0.0402
3	0.0164	0.0166	0.0282	0.0275	0.0418	0.0408
6	0.0178	0.0172	0.0284	0.0277	0.0427	0.0414
24	0.0184	0.0177	0.0285	0.0278	0.0442	0.0427

[a] Previously established factors for bromination at room temperature for 1 our were used.

ndicated by the close agreement between the weights of rubber calculated by these gravimetric factors and the weights obtained by evaporation of the pure rubber benzene extracts. This agreement does not preclude the possibility of obtaining high rubber values by the bromide method from impure benzene extracts. However, the possibility is remote that any nonrubber plant materials are present which are soluble in benzene and also form alcohol-insoluble bromine derivatives. Added antioxidants and solution aids have never interfered.

REAGENTS

Alcohol, A.C.S., absolute
Acetone, A.C.S., analytical reagent grade
Benzene, A.C.S., analytical reagent grade
Trichloroacetic acid, Eastman Kodak Company White Label
Benzene containing 1% trichloroacetic acid
Brominating solution. Dissolve 2 grams of iodine in 100 ml. of carbon tetrachloride, filter through filter paper, and add 5 ml. of bromine to the filtrate.

ANALYTICAL PROCEDURES

Determination of rubber by the bromide method is useful in evaluating the rubber content of benzene extracts of plant tissues, crude rubbers isolated from plants by mechanical or chemical means, and rubber-containing liquors and lattices.

Determination of Rubber in the Organic Solvent Extract of Plant Tissue. An organic solvent extract or the rubber film obtained by evaporating an extract may be analyzed by the bromide procedure.

To analyze a rubber film, redissolve it in benzene by stirring and gentle heating, cool, and make to volume in a volumetric flask with benzene. Choose as the final volume of either the extract or the redissolved film one which will contain 0.5 to 3.0 mg. of rubber per ml. Transfer to a beaker an aliquot containing a minimum of 25 mg. of rubber, and add 1 ml. of the brominating solution for each 10 ml. of the aliquot. Cover the beaker with a watch glass and let stand at room temperature for one hour to permit agglomeration of the particles. At the end of the hour stir the brominated mixture thoroughly with a glass rod, taking special care to loosen any precipitate from the bottom of the beaker. Add absolute alcohol, with constant stirring, in the ratio of 30 ml. of alcohol for each 10 ml. of the brominated mixture. Allow the bromide precipitate to settle for at least one hour and then filter with suction, using a tared Gooch crucible with asbestos pad. An ordinary rubber policeman and alcohol may be used in transferring the bromide and in washing the beaker. Wash the bromide several times with alcohol, dry at 50° C. in vacuo under 5-mm. mercury pressure, cool, and weigh. Calculate the rubber content from the weight of the rubber bromide by the following gravimetric factors: 0.290 for kok-saghyz, 0.292 for guayule, and 0.303 for Cryptostegia.

Determination of Rubber in Rubber Crudes. Prepare the analytical sample of the crude rubber by passing it several times through compounding rolls, finally mill it into a thin ribbonlike sheet, and from this cut or tear narrow strips for moisture and for rubber analysis.

Determine the moisture by drying approximately 2 grams of the crude rubber in vacuo at 50° C. to constant weight (12 hours).

Solution of the sample for rubber analysis may be obtained either by heating it in benzene or by allowing it to stand at room temperature in a 1% solution of trichloroacetic acid in benzene. Although the latter method requires 2 to 3 days for solution, it is more convenient in routine analysis, and in general less time-consuming. Since determination of the benzene-insoluble and acetone-insoluble content of the crude rubber is usually required in addition to rubber hydrocarbon, samples of approximately 0.5 grams are recommended.

Cut or tear approximately 0.5 gram of the milled sheet into fine strips, weigh to the nearest 0.1 mg., and place in a tared 70-ml. centrifuge tube. Add 50 ml. of a 1% solution of trichloroacetic acid in benzene, insert a glass stirring rod, lightly stopper the tube with a plug of cotton, and allow it to stand at room temperature for 3 days. To aid in the complete solution of the rubber, stir two or three times a day during the 3-day interval. Centrifuge to separate the benzene-insolubles and decant the benzene-rubber solution into a 200-ml. volumetric flask. Wash the benzene-insolubles and the centrifuge tube twice with 50-ml. portions of benzene, centrifuging after each wash. Add the benzene washes to the benzene-rubber solution and dilute to 200 ml. with benzene. Determine the rubber in a 20-ml. portion of this solution by the bromide method described in the previous section. Calculate the per cent rubber in the crude by the equation:

$$\% \text{ rubber} = \frac{\text{gravimetric factor} \times \text{weight of bromide (g.)} \times \text{volume of sample (200 ml.)} \times 100}{\text{weight of crude rubber (grams)} \times \frac{100 - \% \text{ moisture}}{100} \times \text{portion used (20 ml.)}}$$

If the percentage of acetone-soluble or resin fraction and the percentage of those materials insoluble in both acetone and benzene are desired, determine the insolubles directly and calculate the acetone-solubles by difference. To determine the insoluble fraction, thoroughly wash the benzene-insoluble residue left in the centrifuge tube with acetone, separate the insolubles by centrifuging, and discard the acetone wash solution. Dry the residue in the tared centrifuge tube for one hour at 110° C. in an air oven, cool, weigh, and calculate the per cent insolubles on a moisture-free basis.

The per cent acetone-soluble fraction is obtained by subtracting the sum of the percentages of moisture-free rubber and insolubles from 100. Since the proposed method is especially suited to the analysis of crude rubber and is applicable to samples containing either large or small amounts of nonrubber nonresin materials, several typical analyses of samples of such rubber from different plants are given in Table V. These show the reproducibility of the method.

Determination of Rubber in Aqueous Liquors Low in Rubber Content. Many types of aqueous rubber-containing emulsions having rubber contents ranging from less than 1 to about 5% may be conveniently analyzed for rubber by the bromide procedure. Benzene extracts of the rubber in such emulsions are most easily prepared in the Waring Blendor. The

Table V. Analyses of Crude Rubber from Different Plants

Plant Source	Sample No.	% Rubber		% Insolubles (Benzene and Acetone)		Acetone-Solubles[a] (Resins)
Guayule	5293	65.0	65.1	15.3	15.3	19.7
	5319	78.6	79.2	5.3	5.3	15.9
	5341	68.2	68.0	10.4	10.3	21.6
	5564	73.8	73.9	7.2	7.3	18.9
	5568	77.4	77.5	1.8	1.8	20.8
Kok-saghyz	7510	80.9	80.9	6.4	6.9	12.5
	7520	86.2	86.1	7.2	7.2	6.7
	7579	77.3	76.8	8.0	8.0	15.0
	8088	79.0	78.7	7.1	7.4	13.9
	8227	77.5	77.5	9.7	9.9	12.7
Cryptostegia	7726	64.5	65.3	1.1	1.0	34.1
	9040	87.4	87.2	5.2	5.3	7.5
	9050	63.1	63.3	28.1	28.1	8.7
	9054	51.3	51.2	40.5	40.3	8.4
	9060	84.6	84.4	5.4	5.3	10.2

[a] Calculated by difference from average of both rubber and insolubles.

persistent emulsions which result when alkali-stabilized rubber liquors are extracted with benzene in the Blendor can be avoided by acidifying the liquor with concentrated hydrochloric acid preliminary to the extraction.

To determine the rubber content, transfer 10 to 50 ml. of the emulsion to a Waring Blendor using a transfer pipet, rinse the pipet several times with water, and combine the rinses with the measured sample, making a total volume of approximately 75 ml. Add sufficient concentrated hydrochloric acid to break the emulsion; usually 5 to 10 ml. are required. Then add about 180 ml. of benzene, churn for 2 minutes, wash the emulsion into a 500-ml. separatory funnel with water, and allow to stand until the aqueous layer appears clear, usually about 8 hours. Draw off and discard the clear aqueous layer.

Transfer the benzene and the interface layers to centrifuge tubes, centrifuge until separation into layers is complete, and transfer the benzene layer to a 400-ml. beaker using a pipet and suction. Rinse the separatory funnel with benzene. Add the rinses to the aqueous residue in the centrifuge tube, mix well, and centrifuge. Again draw off the benzene layer and add it to the original benzene solution in the beaker. Concentrate on a steam bath, transfer, and make to the mark in a volumetric flask of such volume that 20 ml. of the final volume will contain between 10 and 60 mg. of rubber. Transfer a 20-ml. aliquot to a 250-ml. beaker and brominate by the previously described procedure. Should the 20-ml. aliquot of the original solution contain more than 60 mg. of rubber, dilute to the desired concentration and brominate a 20-ml. portion. When the total benzene extract is very low in rubber, it may be reduced to 20 ml. and brominated in its entirety. Calculate the rubber content from the equation:

$$\text{Mg. of rubber/ml.} = \frac{\text{gravimetric factor} \times \text{weight of bromide (mg.)} \times \text{dilution factor}}{\text{ml. of sample}}$$

Determination of Rubber in Latex or Liquor of High Rubber Content. When concentrated rubber liquors (containing more than 5% rubber) and latices are being analyzed, complete extraction of the rubber by benzene cannot be achieved by the Waring Blendor method described above. Instead, benzene is added to the sample, the mixture is acidified, and the water is removed by distillation in a Bidwell-Sterling type moisture apparatus. This results in a water-free solution of the rubber benzene.

Weigh by difference from a glass-stoppered vial that amount the well-mixed sample which contains less than 1.5 grams rubber and transfer to a 500-ml. volumetric (boiling) fla equipped with a 24/40 $\overline{\text{S}}$ outer joint. Acidify the sample with co centrated hydrochloric acid to give an acid concentration about 10%, then add 200 ml. of benzene and attach the flask to Barrett type Bidwell-Sterling moisture test apparatus provid with a condenser. Boil the mixture until all water is remov (water level in the trap remains constant), and a clear benze solution is obtained. Remove the flask from the apparatu cool, and dilute to the mark with benzene. Transfer, with pipet, a 50-ml. aliquot to a 600-ml. beaker and determine t rubber in this aliquot by the bromide method and the followi equation:

$$\% \text{ rubber} = \frac{\text{Gravimetric factor} \times \text{weight of bromide (grams)} \times \text{volume of sample (500 ml.)} \times 100}{\text{weight of sample} \times \text{aliquot (50 ml.)}}$$

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Determination of Alkyl Disulfides in the Absence and Presence of Mercaptans

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Disulfides are reduced to mercaptans by means of amalgamated zinc and titrated amperometrically with silver nitrate. If the relative amount of mercaptan present is not large, the disulfide is determined by difference by titrating portions of the solution before and after reduction. To determine a trace of disulfide in a mercaptan, the mercaptan is removed before the reduction treatment by precipitation as silver mercaptide.

BASED upon the reducibility of alkyl disulfides (*1*, *3*, *5*) to mercaptans and amperometric titration of the resulting mercaptans (*4*) a simple method of determination of disulfides has been developed. Experiments were carried out to find suitable conditions for quantitative reduction and for removal of the mercaptan from the disulfide before reduction.

REDUCTION OF DISULFIDE

Preliminary experiments showed that in order to approach quantitative reduction in a Jones reductor, a temperature of 50° C. was necessary. The zinc amalgam was of a very low concentration (0.02%) in mercury. Under these conditions evolution hydrogen occurred when an acid solution was passed through t reductor. To prevent difficulties encountered with the count flow of liquid and gas, the solution to be reduced was made pass upwards through the zinc in the reductor. A sketch of reductor with a heating jacket, which is easily constructed a satisfies the above specifications, is shown in Figure 1.

Two different samples of *n*-dodecyl disulfide were used wit identical results. At the University of Minnesota, the disulfi was prepared by oxidation of *n*-dodecyl mercaptan with hydrog peroxide in alkaline ethanol solution, followed by recrystallizati from absolute ethanol. The product had a melting point of 32 in agreement with the value of Collin *et al.* (*1*). At the Universit of Illinois, *n*-dodecyl disulfide was prepared by the method Westlake and Dougherty (*6*), in which the Bunte salt prepare from *n*-dodecyl bromide and sodium thiosulfate was oxidize with hydrogen peroxide in aqueous dioxane medium. Th crystals which separated upon cooling and standing were e tracted with dioxane, and the disulfide was precipitated by t addition of water. The product had a melting point of 34–35°, i agreement with the value of 33.5–34° reported by Fore an Best (*2*). It is not certain whether the low results were cause

ıtirely by incomplete reduction, or partly by the presence of ıpurities.

Known amounts of *n*-dodecyl disulfide were dissolved in a 0.3 *N* ɔlution of sulfuric acid in 90% ethanol, and 25-ml. portions of ıe solution, heated to 50°, were allowed to flow through the re-uctor which was maintained at a temperature of 50°, at such a ıte that the disulfide remained in contact with the solution for bout 5 minutes. The reductor was washed with 75 ml. of 0.3 *N* ılfuric acid in 90% ethanol, and the mercaptan was determined ı the resulting solution after addition of an excess of ammonia ɔrresponding to about 0.25 *M* by the amperometric method of olthoff and Harris (*4*). Results obtained with different mounts of disulfide are shown in Table I.

An alternative procedure consisted of adding amalgamated ınc to the acidic disulfide solution (80 ml.) in a 4-ounce screw-ap bottle and rotating it in a thermostat at 50°, usually for 20 inutes. Results with this procedure are given in Table II sing 0.3 *N* sulfuric acid and 1 *N* acetic acid in 90% alcohol as ıe solvent.

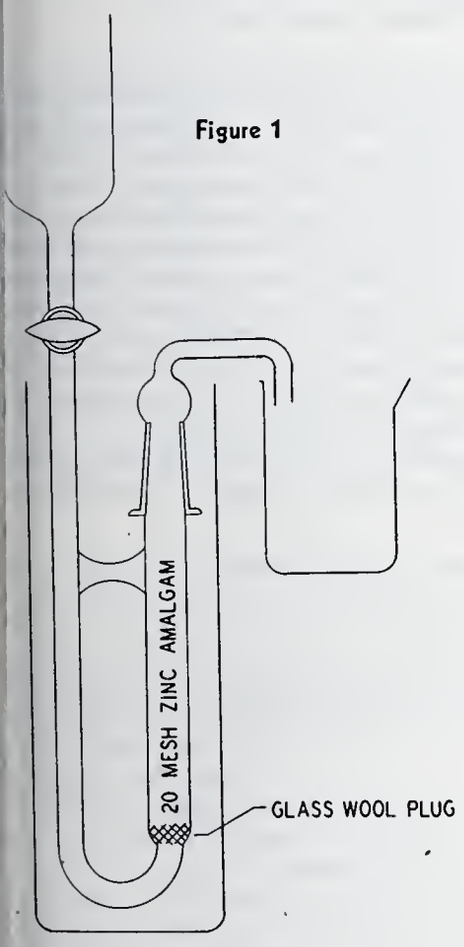
Table I. Reduction of *n*-Dodecyl Disulfide in Jones Reductor

Disulfide Taken *Mg.*	Disulfide Found *Mg.*	Reduction %
40.0	38.3	95.8
	38.4	95.0
	38.3	95.8
20.0	19.1	95.2
	19.0	94.8
	19.1	95.2
10.0	9.05	95.5
4.00	3.90	97.6
2.00	1.96	98.0

Table II. Reduction of *n*-Dodecyl Disulfide in Bottles

Disulfide Taken *Mg.*	Time *Min.*	Disulfide Found *Mg.*	Reduction %
	Using Sulfuric Acid		
15.6	20	14.9	95.5
4.52	60	4.29	94.9
3.12	20	3.03	96.8
3.01	20	2.89	96.1
0.752	60	0.700	93.1
0.312	20	0.202	65.0
0.301	60	0.250	82.9
	Using Acetic Acid		
4.52	20	3.48	76.9
4.52	60	4.20	93.0
3.01	20	2.81	93.3
3.01	60	2.94	97.7
1.51	20	1.49	98.6
0.752	10	0.738	98.2
0.752	30	0.720	95.8
0.752	60	0.704	93.6
0.301	10	0.304	101
0.301	20	0.307	102
0.150	10	0.179	119
0.150	10	0.188	125

Figure 1



The use of acetic instead of sulfuric acid was found beneficial for small amounts of disulfide (less than 4 mg.). If large quantities of disulfide were present the reduction was slower in acetic acid medium, while with very small amounts (less than 0.3 mg.) the reduction was incomplete using sulfuric acid. Changing the concentration of acetic acid did not appreciably affect the rate or completeness of reduction.

SEPARATION OF MERCAPTAN FROM DISULFIDE

For the determination of small amounts of disulfide in the presence of large amounts of mercaptan it is necessary to remove the mercaptan, since the mercaptan cannot be titrated with sufficient accuracy to allow the disulfide to be determined by difference. The mercaptan can be removed by adding an exactly equivalent amount of silver nitrate and filtering off the silver mercaptide. An appreciable excess of silver leads to low results for disulfide, since silver mercaptide is precipitated during the reduction step and is not completely reduced to mercaptan. Using the amperometric end point with a sufficiently sensitive galvanometer, it is easily possible to adjust the excess silver concentration to a very small quantity.

Experiments involving the removal of a 1000-fold excess of *n*-dodecyl mercaptan from known amounts of *n*-dodecyl disulfide gave an excellent recovery of disulfide after reduction and titration. Using mixed tertiary mercaptans, some difficulty was encountered in filtration of the silver mercaptide, since the mercaptide mixture was a gummy liquid at room temperature. Working at a temperature of 0° throughout the precipitation and filtration, however, gave satisfactory results.

DETERMINATION OF DISULFIDE IN PRESENCE OF MERCAPTAN

A. Jones Reductor Method. Determination by Difference. *Preparation of Amalgamated Zinc.* Etch 100 grams of 20-mesh granulated zinc by stirring with 6 *N* hydrochloric acid for one minute. Add 27 mg. of mercuric chloride, and after stirring for a minute, wash the zinc with distilled water. One hundred grams of zinc are sufficient for a reductor of a convenient size.

Incomplete reduction is encountered if the reductor is not completely cleaned by acid rinsing. A 10 *N* sulfuric acid solution was found to be satisfactory for the rinsing. The reductor should be checked before every run or series of runs by reducing a standard disulfide solution and determining the amount reduced. If the reduction remains slow the reductor should be filled with freshly prepared zinc amalgam.

Reduction of Disulfide. Using 90% ethanol–10% water 0.3 *N* in sulfuric acid as the solvent, allow the solution at 50° to run through the reductor at such a rate that the disulfide remains in contact with the zinc for about 5 minutes. Wash the reductor with 75 ml. of the solvent.

Determination of Mercaptan. Neutralize the solution with concentrated ammonium hydroxide, using litmus paper as an indicator. Add 2 ml. in excess, and titrate amperometrically with silver nitrate. If mercaptan was present initially, titrate a second portion of the solution without reduction. The difference is a measure of disulfide present.

B. Bottle Method. Removal of Mercaptan. For the determination of a trace of disulfide in mercaptan, dissolve a 0.1- to 0.2-gram sample of mercaptan in 80 ml. of absolute ethanol and add concentrated ammonium hydroxide (1.2 ml.) until the solution is about 0.25 *M* in ammonia. Titrate the solution amperometrically with 0.05 *N* silver nitrate to a very slight excess of silver. A highly sensitive current-measuring instrument such as a wall-type galvanometer is recommended. If necessary, adjust the excess silver by the addition of 0.005 *M* mercaptan in alcohol and 0.002 *N* silver nitrate until the excess of silver, as indicated by the current, corresponds to about 0.1 ml. of 0.002 *N* reagent.

Filter the solution without suction and wash the precipitate thoroughly with small portions of alcohol. Add 5 ml. of glacial acetic acid. Add 10 to 20 mg. of amalgamated zinc prepared as described in Procedure A to the solution in a 4-ounce screw-cap bottle and rotate in a 50° thermostat for 20 minutes.

Filter the solution again, taking care that no small pieces of zinc amalgam pass into the filtrate. Add 7 ml. of concentrated ammonium hydroxide and titrate with 0.002 *N* silver nitrate to the usual amperometric end point.

ACCURACY AND SENSITIVITY

The accuracy of the method is limited by the degree of reduction of the disulfide by the amalgamated zinc. Using either method the results are about 5% low if the amount of disulfide is in the optimum range. The results are reproducible to 2% relative on a given sample in the optimum range.

Method B has proved successful in detecting 0.05% disulfide in mercaptan and in making quantitative estimation of disulfide in amounts from 0.1 to 1% or more.

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Higher Aliphatic Acid Chlorides

Estimation of Yield in the Preparation of Lauroyl Chloride

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A titration procedure for estimation of yield in the preparation of lauroyl chloride has been found equally applicable for other fatty acid chlorides containing 12 to 18 carbon atoms. Using this procedure, the effect of the ratio of lauric acid concentration to thionyl chloride concentration on the yield of lauroyl chloride was determined. The titration method is applicable to the determination of fatty acid chloride content in reaction mixtures or in distilled products. By the use of this procedure, it was found that undistilled acid chlorides prepared from the corresponding fatty acids and thionyl chloride are sufficiently pure for most synthetic work.

IN THE course of an investigation of derivatives of higher fatty acids it was necessary to prepare relatively large quantities of lauroyl chloride. When the acid chloride was prepared by the action of thionyl chloride on lauric acid and the yield determined by distillation, it was found significantly lower in the case of reactions involving 1 mole of lauric acid than in identical experiments employing only 0.1 mole of the acid. During the distillation of larger runs, large amounts of hydrochloric acid were evolved.

Among other explanations for lowered yield and evolution of acidic fumes, it seemed possible that the reaction of lauroyl chloride with unreacted lauric acid might take place:

$$C_{11}H_{23}COCl + C_{11}H_{23}COOH \longrightarrow (C_{11}H_{23}CO)_2O + HCl \quad (1)$$

The conditions of distillation (flask temperature approximately 130° C. at 2 mm. of mercury) might favor this reaction. It is apparent, further, that if the initial conversion amounts to 90%, the unconverted acid is capable of reaction with a sufficient quantity of acid chloride to reduce the apparent yield on distillation to 80%, the anhydride remaining behind in the residue. Ketene formation, followed by polymerization, may also be a yield-reducing factor. The polymerized products would not be distillable. An experiment in which the reaction mixture was refluxed under vacuum for a prolonged period showed that the amount of acid chloride present was reduced during the heating period. If small quantities of acid chloride are involved, distillation may be carried out with sufficient rapidity to minimize yield-reducing side reactions.

The high yields obtained when small quantities are involved, together with the demonstrated reduction of yield during slow distillation, suggested the probability that lauric acid chloride could be prepared in sufficiently high yield to make distillation unnecessary. In order to prove this, it was necessary to develop an analytical procedure independent of distillation or prolonged heating.

Several derivatives of lauroyl chloride were prepared which might lend themselves to quantitative recovery. Quantitative separation of these derivatives from unreacted fatty acid was difficult, however, and an indirect method based upon the formation of amide from lauroyl chloride with subsequent dete mination of the unreacted lauric acid proved to be more sati factory. Since the completion of this work Bauer (*2*) has d scribed an analytical procedure based upon anilide formatio While the principles involved in these two methods are simila the procedure described in the present paper permits accura estimation of fatty acid chloride in the presence of extraneo matter (in addition to free fatty acid) and the technique is mo readily adaptable to plant control. Caspari (*3*) and Krafft an Stauffer (*4*), have prepared lauramide by the action of lauro chloride on aqueous ammonia. Aschan (*1*) investigated t preparation of various amides by this method.

When a mixture of lauric acid and lauroyl chloride is stirre rapidly into a tenfold excess of aqueous ammonia, the followi reactions take place:

$$C_{11}H_{23}COCl + 2NH_3 \longrightarrow C_{11}H_{23}CONH_2 + NH_4Cl \quad (2)$$

$$C_{11}H_{23}COOH + NH_3 \longrightarrow C_{11}H_{23}COONH_4 \quad (3)$$

Because of the emulsifying properties of the ammonium la rate, the reaction mixture is a soapy suspension. Upon acidific tion, the ammonium laurate is converted into lauric acid while t amide remains unchanged. After extracting the lauramid lauric acid mixture with ether, the amount of lauric acid in t mixture may be determined by titration according to the pr cedure for determination of free fatty acid.

The total lauric acid content may be accurately determined b saponification of the reaction mixture with excess aqueous sodiu hydroxide, followed by acidification, extraction, and titratio The total content of lauric acid determined by this procedure w found to be in excellent agreement with the known lauric ac content based upon the amount of lauric acid employed and t total weight of the reaction mixture.

From the above determinations, the per cent of free fatty ac and the per cent of total fatty acid are obtained. Using the data the per cent yield and per cent purity of the lauroyl chlori can be calculated.

Using this procedure, when a reaction mixture resulting fro the action of thionyl chloride upon lauric acid was heated 150° C. and 5 mm. of mercury, the apparent conversion w found to decrease from an initially determined value of 96 to 85 after 2 hours.

The applicability of this procedure for studying the effect varying the proportion of thionyl chloride to lauric acid on t yield of lauroyl chloride was determined by a series of experimen in which the reaction mixtures were analyzed by this meth (Table I and Figure 1).

The values found for yield are apparently independent of t relative proportions of lauric acid and lauroyl chloride, where

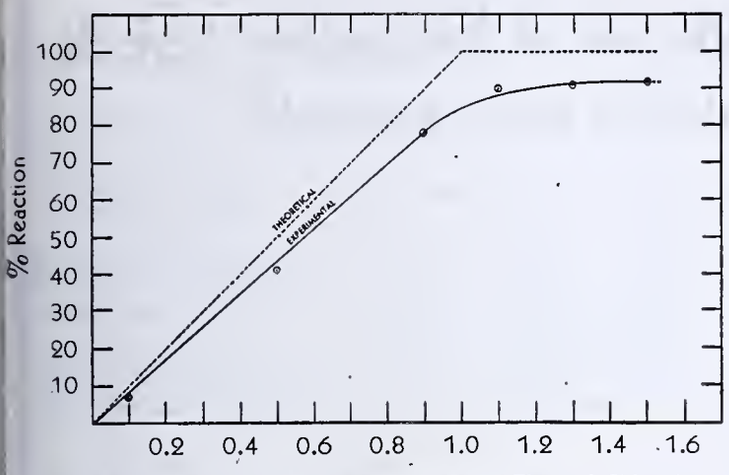

Figure 1. Effect of Varying Proportion of Thionyl Chloride

Moles of thionyl chloride per mole of lauric acid

n the presence of a large proportion of unreacted lauric acid the auroyl chloride present cannot be distilled quantitatively.

When distilled lauroyl chloride was analyzed by this method, he composition was in excellent agreement with that found by hloride determination. A product from the reaction of 1 mole of auric acid and 1.2 moles of thionyl chloride, when analyzed prior o distillation, showed 96% conversion to lauroyl chloride. By listillation, 90% of the theoretical amount of distillate was collected. Upon analysis of the distillate by chloride determination, however, the chloride content was found to amount to only 2% of the calculated. A similar result was obtained when the titration procedure was employed. Thus, the actual percentage yield after distillation was 83% and the free lauric acid content of he distilled acid chloride was no less than that of the undistilled reaction mixture from which it was obtained.

EXPERIMENTAL

The lauric acid used in all experiments was obtained by careful fractionation of coconut fatty acids through a 25-cm. Widmer column. The portion used was collected at 145° to 150° C. at 3 mm. of mercury. It had a neutralization equivalent of 199.5 (theoretical 200) and melted at 43–47° C. The thionyl chloride was the fraction boiling at 72–76° C., obtained by distillation of a commercial product.

Lauroyl chloride was prepared by the method of Ralston and Selby (*5*).

A. Determination of Unreacted Lauric Acid (Free Fatty Acid) in Crude or Distilled Acid Chloride. Ten grams of acid chloride were added slowly with cooling and strong agitation to 125 ml. of 6% ammonium hydroxide. The suspension obtained was allowed to stand with intermittent stirring and cooling at room temperature for 20 minutes. Hydrochloric acid, 10 *N*, was then added dropwise with stirring and cooling until the methyl orange end point was reached.

One hundred and fifty milliliters of a 2 to 1 mixture of ethyl ether and ethanol were added, and the mixture was transferred to a 500-ml. separatory funnel, the reaction vessel being washed with repeated small portions of ethanol. The aqueous layer was withdrawn, and the extracted material washed repeatedly with 50-ml. portions of saturated sodium chloride solution until the last wash-ing became alkaline to phenolphthalein on the addition of a single drop of 1 *N* sodium hydroxide.

The solvent solution was transferred to a 500-ml. flask, and the separatory funnel washed with 100 ml. of ethanol in small portions. The extract, together with the combined ethanol washings, was titrated to the phenolphthalein end point with 1 *N* sodium hydroxide.

B. Determination of Total Lauric Acid (Total Fatty Acid) in Crude or Distilled Acid Chloride. Ten grams of acid chloride were added with cooling and stirring to 125 ml. of 10% sodium hydroxide solution. The resulting soapy solution was allowed to stand at room temperature for 20 minutes, and acidified to methyl orange as before with 10 *N* hydrochloric acid.

Determination of Yield of Lauroyl Chloride by Distillation and by Titration. Lauroyl chloride was prepared by the reaction of 1.2 moles of thionyl chloride and 1.0 mole of lauric acid. The total weight of the crude reaction mixture was 224 grams. This crude product was analyzed by procedures A and B, giving 4% free fatty acid, 89% total lauric acid, a percentage yield of 96%, and a lauroyl chloride content of 92%.

One hundred and ninety grams of the reaction mixture (84% of the total weight) were distilled from a Claisen flask, 165 grams of distillate being collected at 105–115° at 2 mm. of mercury. If the distillation produced practically 100% lauroyl chloride, the theoretical yield would be 218.5 × 0.84 or 183. Therefore 165 grams correspond to an apparent percentage yield of 90%. Analysis of the distilled product by the method described above gave 7% free fatty acid, and 91% total fatty acid. This is equivalent to 92% lauroyl chloride content and the percentage yield after distillation becomes 83%. It is evident that the crude reaction product, lauroyl chloride content 92%, contains the same percentage of lauroyl chloride as the product obtained by distillation. It appears from these results that distillation of the crude reaction products is not advantageous, since the quality of the product is not changed and the yield is decreased by 13%.

A chloride determination of the distilled product gave 14.9% chlorine. This value, compared with the calculated value of 16.3%, indicates a lauroyl chloride content of 91%. This is in close agreement with the result obtained by the titration method (92% lauroyl chloride content).

Effect of Prolonged Heating of Lauroyl Chloride. Lauroyl chloride was obtained in 96% yield by the action of 1.2 moles of thionyl chloride on 1.0 mole of lauric acid. Analysis of the crude product by procedures A and B indicated a lauroyl chloride content of 92% (TFA = 89%; FFA = 4%).

One hundred and eighty grams of reaction mixture were maintained at 150° C. and 5 mm. of mercury for 2 hours, then again analyzed by procedures A and B; the lauroyl chloride content was found to be 84% (TFA = 91%; FFA = 14%), the apparent percentage yield after heating being 85%. The decrease in percentage yield as indicated by this experiment was comparable with that observed during a distillation carried out under similar conditions.

DISCUSSION

The precision of the titration method, as indicated by a large number of duplicate determinations, is ±1%.

The titration method does not differentiate clearly between acid chloride and acid anhydride, as the latter, if present, is also capable of reaction with ammonia to form amide. If the acid chloride is to be employed in acylation reactions, however, this is not serious. Further, it is obvious that a reaction mixture which shows 96% acid chloride by titration must contain a minimum of 92% acid chloride and a maximum of 8% anhydride.

This procedure has been applied to the determination of yield of both saturated and unsaturated aliphatic acid chlorides containing 12 to 18 carbon atoms. Oleic acid chloride and saturated acid chlorides containing up to 16 carbon atoms can be analyzed by this procedure, giving results of the same accuracy as those obtained with lauric acid chloride. In the analysis of stearic acid chloride, stearic acid and stearamide tend to crystallize from ether at room temperature, but it was found that this did not significantly influence the ease of removal of excess mineral acid or the accuracy of the results.

Table I. Preparation of Lauroyl Chloride

(With varying amounts of thionyl chloride, % reaction)

Lauric Acid	$SOCl_2$	FFA in Crude Product		TFA in Crude Product		Yield of Crude Product		Reaction	
		Calcd.	Found	Calcd.	Found	Calcd.	Found	Theoretical	Experimental
Mole	*Mole*	*Grams/10 grams*		*Grams/10 grams*		*Grams*		%	%
0.5	0.05	8.9	9.3	10.0	9.9	101	100	10	7
0.5	0.25	4.7	5.6	9.6	9.4	105	104	50	41
0.5	0.45	0.9	2.0	9.3	9.1	108	108	90	78
0.5	0.55	0.0	0.9	8.9	9.0	114	112	100	90
0.5	0.65	0.0	0.7	8.3	8.0	126	120	100	91
0.5	0.75	0.0	0.6	7.7	7.6	139	130	100	92

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Thiocyanate Complex as a Means of Extracting Cobalt

Before Its Microdetermination by Other Methods

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THE colored complex of cobalt with nitroso R salt forms the basis of several analytical procedures for the microestimation of cobalt. Particularly in the case of biological samples, some separation of cobalt from other metals is a necessary preliminary to its estimation. Sylvester and Lampitt (*7*), for example, effected this preliminary separation by precipitating the cobalt (together with iron and copper) with α-nitroso-β-naphthol. Marston and Dewey (*2*) used dithizone extractions at pH 5 and pH 8, the first to separate other heavy metals and the second to extract cobalt.

Since few metals other than cobalt react with ammonium thiocyanate to form a complex salt that can be extracted with organic solvents, ammonium thiocyanate suggested itself as a useful reagent to effect a preliminary separation of cobalt. The blue complex, $(NH_4)_2Co(SCN)_4$, is itself used for the colorimetric estimation of cobalt (*5*, *8*), but the color is not intense enough to be used for the small amounts of cobalt in biological samples. Iron, nickel, copper, and zinc as well as cobalt form complex thiocyanates that are at least partly extracted by organic solvents. However, the iron complex is not very soluble in solvents containing ether (*3*). Furthermore, nickel, copper, and zinc do not interfere with the final estimation of cobalt as the nitroso R salt complex (*2*).

The present investigation had a twofold object: to determine the best conditions (solvent composition, pH, and ammonium thiocyanate concentration) for extracting the cobalt thiocyanate complex, and to determine whether the extract could be used for the estimation of cobalt as the nitroso R salt complex. The authors were led finally to the following recommended procedure, which was used for all the cobalt recoveries described below, except for the obvious modifications required for investigating the effect of varying the conditions of the extraction.

The work reported was carried out during 1939 and would have been completed by a series of actual tests on biological samples but for the outbreak of the war. It is unlikely that the cobalt project will be resumed, but the authors believe that the results obtained will be sufficiently useful to other workers to justify publication.

RECOMMENDED PROCEDURE

a. Dissolve the sample (containing, for convenience, 10 to 20 micrograms of cobalt) in hydrochloric acid, so that the resulting solution contains an excess of about 5 ml. of 6 *M* hydrochloric acid.

b. Add 20 ml. of 60% ammonium thiocyanate. The solution will normally be colored red at this stage owing to iron in the sample or in the reagents. Buffer the solution by adding 1 *M* ammonium citrate until the red ferric thiocyanate just disappears. Dilute with water to 50 ml. and add 4 ml. of ether to saturate the solution.

c. Extract the buffered aqueous solution with three successive 20-ml. portions of a mixture of 35% amyl alcohol and 65% ether. Cobalt enters the nonaqueous phase as $(NH_4)_2Co(CNS)_4$, while the extraction of ferric iron is prevented by the ammonium citrate.

d. Shake the combined extracts with two 20-ml. portions of 2 *M* ammonium hydroxide, thus returning the cobalt to the aqueous phase.

e. Evaporate the combined ammonia extracts to dryness. Add 20 ml. of 6 *M* nitric acid to destroy thiocyanate, and evaporate to dryness. Neutralize the resulting ammonium hydrogen sulfate by adding 2 *M* ammonium hydroxide and evaporate to dryness.

f. From this point the procedure is essentially that of Marston and Dewey (*2*). To the residue add 5 ml. of water, 5 ml. of ammonium citrate, and 0.5 ml. of nitroso R salt reagent. Heat the solution on a water bath for 5 minutes, add 5 ml. of concentrated nitric acid, and heat 5 minutes longer. After cooling, add 2 ml of aqueous bromine, and remove excess bromine by boiling Determine the cobalt content by comparison with standards containing known amounts of cobalt.

g. The comparison standards are prepared from the required amount of standard cobalt sulfate solution and ammonium sulfate. Evaporate the mixture to dryness and treat the residue as in *f*.

REAGENTS

AMMONIUM THIOCYANATE SOLUTION, 60%. Dissolve 42 grams of ammonium thiocyanate in 280 ml. of water. Thoroughly extract the solution with a 0.1% solution of dithizone in chloroform, and remove the excess dithizone by washing with chloroform, and the excess chloroform by washing with amyl alcohol The proper pH for the dithizone extraction may be found by adding ammonia to the ammonium thiocyanate until, on adding cobalt to a small sample of the solution, the cobalt is easily extracted again with dithizone.

AMMONIUM CITRATE, 1 *M*. Dissolve 210 grams of citric acid in 400 ml. of water, add 200 ml. of concentrated ammonia, and cool the solution. Add more ammonia until the solution is alkaline to phenolphthalein. Adjust the volume to 1 liter, and purify the solution as above with dithizone.

AMMONIUM SULFATE, 10%. Dissolve 100 grams of ammonium sulfate in 900 ml. of water. Purify the solution with dithizone as before.

AMMONIUM HYDROXIDE, 2 *M*. Redistill concentrated ammonia in Pyrex and dilute to the required extent.

HYDROCHLORIC ACID, 6 *M*. Dilute concentrated hydrochloric acid to the required extent and distill in Pyrex.

NITRIC ACID, CONCENTRATED. Twice distill pure nitric acid in Pyrex.

BROMINE, 0.2 *M*. A saturated solution of bromine (analytical reagent grade) in water.

NITROSO R SALT REAGENT. A 1% solution in water.

STANDARD COBALT SOLUTIONS. Solutions of cobalt sulfate in water containing 1 and 2 micrograms of cobalt per ml.

Table I. Effect of Solvent Composition, Using Ferric Thiocyanate as Indicator

(Volume of aqueous phase in each case was 50 ml., and it contained 10 micrograms of cobalt, 20 ml. of 60% ammonium thiocyanate, 5 ml. of 6 *M* hydrochloric acid, and sufficient ammonium citrate just to discharge red color of ferric thiocyanate. Solvent phase had a volume of 20 ml.)

Composition of Solvent, Amyl Alcohol *% by Volume*	Cobalt Recovered after Single Extraction %
31	81
33	83
34	86
36	85
37	82
39	75

OPTIMUM COMPOSITION OF SOLVENT

Grahame and Seaborg (*1*) have stated that the partition coefficient of $(NH_4)_2Co(SCN)_4$ between amyl alcohol and an approximately 20% aqueous solution of ammonium thiocyanate is about 10. Rosenheim and Huldschinsky (*3*) recommended a mixture of ether and amyl alcohol in the ratio 25 to 1 for the separation of cobalt and nickel. Since the use of ether has the advantage that the solubility of ammonium thiocyanate in the nonaqueous phase is lowered, it was decided to study different mixtures of amyl alcohol and ether to find the optimum composition. Preliminary trials indicated that the best extraction of cobalt was from aqueous solutions buffered to a pH of between and 4. A series of solvent mixtures was then tested with aqueous solutions buffered with ammonium citrate to be just acid t

ıble II. Effect of Thiocyanate Concentration and pH on Extraction of Cobalt

Aqueous phase contained 20 micrograms of cobalt in each case and was turated with ether. Solvent phase in each case consisted of 20 ml. of a mixture of 35% amyl alcohol and 65% ether.)

	Reagents Added to Aqueous Phase					
	Ammonium thiocyanate, 60%	Hydrochloric acid, 6 *M*	Ammonium citrate, 1 *M*	Water to make	Apparent pH of Aqueous Phase	Cobalt Recovered after Single Extraction
	Ml.	*Ml.*	*Ml.*	*Ml.*		%
eries I	10	1	10	60	5.4	0
	10	2	10	60	4.8	5
	10	3	10	60	4.1	16
	10	4	10	60	3.4	32
	10	5	10	60	2.8	27
eries II	20	2	10	60	4.8	59
	20	3	10	60	4.1	72
	20	4	10	60	3.4	85
	20	5	10	60	2.8	84
eries III	20	2	4.1	65	4.0	78
	20	4	9.0	65	3.8	81
	20	6	13.9	65	3.8	87
	20	8	18.6	65	3.7	83
	20	10	22.7	65	3.7	88

romophenol blue (pH = 3). In each case the aqueous solutions ere saturated with ether (4 ml. in 50 ml. of aqueous solution) fore adding the extracting solvent. The best extraction, as termined by following the analytical procedure outlined above, as obtained with a solvent containing about 35% of amyl alcohol y volume.

After the method of using ferric iron as an indicator had been veloped (see below), a further test was made of solvents in this mposition range. The result is shown in Table I. It was con-rmed that about 34% amyl alcohol is the optimum composition, 5% of the cobalt being removed from the aqueous phase in a gle extraction.

The authors used in each case a single extraction with definite lumes of aqueous phase and extracting solvent. This was done liberately to make it possible to calculate approximate partition efficients if necessary, and to ensure strictly comparable results. hen the results are applied in an analytical method, it is easy determine how many extractions are needed for a desired re-very. Using optimum conditions of 86% recovery in one ex-action, it is seen that two successive extractions would remove %, and three extractions 99.7%, of the cobalt in the aqueous ıase.

The concentration of ammonium thiocyanate in the aqueous ıase was 24%, and the partition coefficient for the complex iocyanate under these conditions is about 15. While the ex-action efficiency is not unduly sensitive to variations in the mposition of the solvent, the authors' optimum composition is r from that recommended by Rosenheim and Huldschinsky (*3*).

EFFECT OF pH AND THIOCYANATE CONCENTRATION

Experiments to study the effect of varying the pH and the iocyanate concentration in the aqueous phase are outlined in able II, Series I and II.

In each case the aqueous phase contained 20 micrograms of balt in 60 ml., and was saturated with ether before shaking ith the extracting solvent. The pH was varied by altering the nount of hydrochloric acid present, and was measured after the balt had been removed by the extracting solvent. A quinhy-one electrode was used, and no attempt was made to correct r the salt effect resulting from the very high electrolyte con-ntration. The pH values are therefore "apparent" values only, d have relative, if not absolute, significance. The cobalt re-vered in a single extraction was determined by carrying out the alytical procedure outlined above.

A pH in the neighborhood of 3.4 is seen to be the best, and a mparison of Series I and II shows the expected marked effect of tering the thiocyanate concentration. The extraction is rea-nably good in Series II, where 60 ml. of aqueous phase contain 20 ml. of 60% ammonium thiocyanate. Using half this concentration as in Series I, the amount extracted is too small to be useful in an analytical method.

SUPPRESSION OF FERRIC IRON AND USE AS INDICATOR

Ferric iron is invariably present at much greater concentration in biological samples than is cobalt, and it is also normally present in small quantities in reagents that have been purified with dithizone, which does not form an iron complex. It is therefore necessary to suppress the ferric thiocyanate complex in order to prevent interference with the cobalt determination. Sodium pyrophosphate has been suggested for this purpose (*6*); but the authors found ammonium citrate more effective. It was also noticed, in the experiments comprising Series I and II, that the addition of ammonium citrate as a buffer caused the red color of the ferric thiocyanate complex (resulting from the iron in the authors' reagents) to disappear at a point very close to the optimum pH for the extraction.

Series III, Table II, records experiments performed to find whether ferric iron could be used as an indicator. Solutions containing 20 micrograms of cobalt were treated with ammonium thiocyanate and varying amounts of hydrochloric acid. Ammonium citrate was then added in each case until the red ferric thiocyanate had just disappeared. The volumes were made up to a convenient, uniform value, and the cobalt was extracted and determined as before. In each case the extraction was good, so that the cobalt in the aqueous phase could be removed practically quantitatively by two or three extractions. The use of ferric iron as an indicator is therefore possible, and it has an advantage over organic indicators in that the latter are carried through the procedure and may interfere with the final color comparison.

REMOVAL OF THIOCYANATE

Ammonium thiocyanate is slightly soluble in the mixture of ether and amyl alcohol, and if it is still present when the excess nitroso R salt is being nitrated, there is a violent reaction between the nitric acid and the thiocyanate, with the formation of a yellow precipitate which is probably isoperthiocyanic acid (*4*). Using the solvent of optimum composition for the extraction, the authors found that 6 *M* nitric acid removed the thiocyanate satisfactorily in stage *e* of the procedure described above. With more concentrated acid, the reaction was too violent.

CONCLUSIONS

In the extraction of the cobalt thiocyanate complex from aqueous solutions containing ammonium thiocyanate and small quantities of cobalt, the most suitable mixture of ether and amyl alcohol is one containing 35% by volume of amyl alcohol, providing the aqueous phase is first saturated with ether.

If the aqueous phase of volume 50 ml. contains 10 to 20 micrograms of cobalt, and if the ammonium thiocyanate concentration is at least 20%, 86% of the cobalt can be removed by a single extraction with 20 ml. of the above solvent if the pH is in the region of 3.5. This corresponds to a partition coefficient of 15 between the solvent phase and the aqueous phase. Two extractions would therefore remove 98%, and three extractions 99.7%, of the cobalt in the aqueous phase.

If the aqueous phase of volume 50 ml. contains 5 ml. of 6 *N* hydrochloric acid, the addition of ammonium citrate until the red color of ferric thiocyanate just disappears not only prevents interference by iron, but at the same time gives the correct pH for the optimum extraction of the cobalt thiocyanate complex, thus dispensing with the need for an organic indicator.

The cobalt thiocyanate complex can be back-extracted from the organic solvent by 2 *M* ammonia, yielding a residue which, after destroying the thiocyanate with 6 *M* nitric acid and neutralizing the resulting ammonium hydrogen sulfate, can be used

for the estimation of cobalt by the nitroso R salt method of Marston and Dewey.

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Small Glass Center-Tube Fractionating Column

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An improved small glass center-tube fractionating column which can be easily reproduced is described and construction details are given. The fractionating section, which is surrounded by a silvered vacuum jacket, is 30.5 cm. long and consists of an 8-mm. inside diameter outer tube and a 6.5-mm. outside diameter center tube. Above the fractionating section a distributor is used which distributes the reflux from the head half to the outer tube and half to the center tube. The column is equipped with an automatic take-off valve. It has an efficiency of over 75 theoretical plates with an H.E.T.P. of less than 0.4 cm.

THERE is always a need in the laboratory for a fractionating column which is capable of efficiently separating small quantities of close-boiling liquids. To be of practical value such a column must have a low operating holdup, must be easily reproduced from readily available materials, and must be easily operated with a minimum amount of attention. A center-tube column previously described (*5*, *6*) was found to fill all these conditions except that it is not easily reproduced.

This article deals with a fractionating column that is similar to the one previously described but includes several improvements in design and construction technique which make it easily reproduced and increase its operating efficiency. The improvements include a reflux distributor, a method of accurately spacing the center tube, and an automatic take-off system. It has an efficiency under conditions of total reflux of over 75 theoretical plates with an H.E.T.P. of less than 0.4 cm. for a 30.5-cm. (1-foot) fractionating section. The column can be used not only for micro work but for work involving larger quantities of liquid.

CONSTRUCTION

The following procedure was used to construct the column shown diagrammatically in Figure 1.

A tube of 6.5 ± 0.1 mm. outside diameter was carefully selected for straightness and uniformity from stock. Capillary tubing was used, since it was found to be more satisfactory in meeting these requirements than either regular tubing or rod. One end of this tube was drawn down and sealed to an 8-mm. ball. Troughs which were made by cutting 7-mm. outside diameter tubing in half with a glass saw were sealed on opposite sides of the ball just below the horizontal center and tilted downward at an angle of 15° from the horizontal. The ends of the troughs were cut at an angle of 30° from the vertical and at right angles to the length. Slots of 1 × 3 mm. were cut into the ends of the troughs. Glass projections to be used for supports were sealed to the tube just below the ball. This assembly was made to fit within 25-mm. outside diameter tubing with a clearance of approximately 1 mm. between the wall and the ends of the troughs and projections with the ball centered.

The bottom of the 6.5-mm. outside diameter tube was drawn down to 2-mm. outside diameter and left extended for 5 cm. (2 inches) with a small needle eye at the end. The length of the tube between the drawn-down ends was 30.5 cm. The tube was wrapped at a 6-mm. pitch with copper wire, which had been stretched to an approximate outside diameter of 0.75 mm., making use of the needle eye and the glass projections to hold the ends of the wire. A 30.5-cm. length of Ace Trubore tubing (Pyrex) of 8 ± 0.01 mm. inside diameter was flared out slightly at both ends. A 25-mm. outside diameter tube was sealed to one end of it. The tube with the wire wrapping was inserted through the large tube into the Trubore tubing by rubbing the wire down carefully with fine emery cloth to obtain a snug fit without any binding. The wire was loosened from the needle eye and cut back to the Trubore tubing. The end of the center tube was drawn down to eliminate the needle eye and was bent over to the side. An 18-mm. outside diameter tube was sealed to the other end of the Trubore tube, care being exercised to avoid constricting the annular space. The bent end of the center tube was sealed to the 18-mm. tube. A drip tip was ring-sealed above the ball with a clearance of about 2 mm. between the tip and the ball. The glass projections were sealed to the wall.

The jacket, bottom drip tip and joint, head assembly, and thermowell, which had a thin wall at the bottom, were added in the order named. The column was annealed. During the annealing, air was drawn slowly through the column to ensure even heating and prevent differential stresses. The copper wire was removed with aqua regia and nitric acid. The meniscus in the annular space was tested for flatness as a check on the uniformity of the spacing by raising and lowering a leveling bulb which was connected to the bottom of the column and contained first water and then a hydrocarbon.

The following evacuation procedure was used after silvering the jacket. The column was placed in an oven and sealed to a system comprising a liquid air trap, a two-stage fractionating pump (Distillation Products Co.), and a Welch Duo-Seal pump. Evacuation was started. The temperature of the column was brought up slowly to 300° C. while air was drawn through the annular space. The evacuation was continued at 300° C. until the pressure was down to 10^{-6} mm. of mercury. The heat was then cut back and the column allowed to cool to approximately 125° C. Evacuation was continued at this temperature until a pressure of 4×10^{-7} mm. of mercury or lower was obtained as measured by an ionization gage unit (Type HG-200, Distillation Products Co.) and then the column was hermetically sealed. The finished column was allowed to cool slowly to room temperature. It was insulated with 1-inch magnesia pipe lagging.

The critical dimensions in the column are those pertaining to the distributor and to the annular clearance between the center tube and the outside tube. The 2-mm. clearance between the drip tip and ball of the distributor gives a liquid contact which spreads the reflux to the column evenly across the ball, with the result that approximately half of it flows down the troughs to the outside wall and the remainder flows down the center tube. The clearance and slots at the ends of the troughs permit a liquid contact to the outside wall. It is not necessary to use Trubore tubing for the outside tube to obtain the desired annular clearance if sufficient care is exercised in selecting a tube of uniform diameter from stock. For general use, however, Trubore tubing is recommended. A head assembly half the size of the one shown

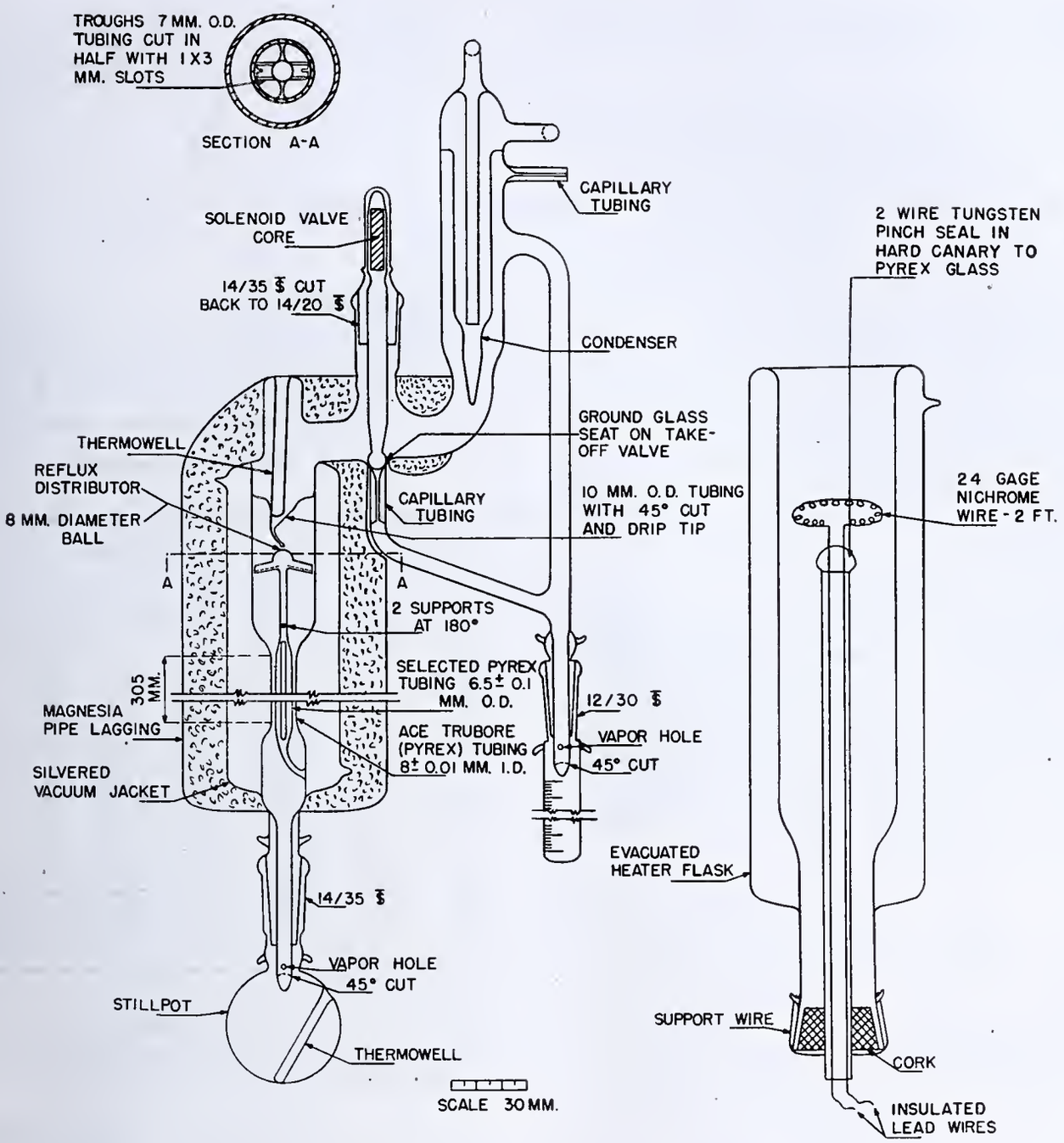

Figure 1. Diagram of Fractionating Column

n Figure 1 has been found to be satisfactory on an otherwise du-plicate column.

The spherical end of the take-off valve was made in a brass nold. The female impression was made with a ball bearing ıttached to a rod. The ground surface was obtained with FFF Carborundum and polished with cerium oxide.

An iron-constantan thermocouple was cemented in the thin-valled thermowell by the method suggested by Baker (*1*), using Technical B copper cement obtained from the Weinbaum Den-al Supply Company (220 West 42nd St., New York, N. Y.). This cement gives good heat conductivity to the thermocouple ınd permits accurate temperature readings.

The evacuated (unsilvered) heater flask was constructed as shown in Figure 1.

OPERATION

The operation of the column requires a minimum amount of ıttention after it has been started and equilibrium conditions have been reached. It consists of periodically measuring the temperatures in the head and still pot, the boilup rate, and the volume or weight of the distillate. Various fractions are collected by changing the product receiver as desired. The take-off rate being slow, the product receiver does not have to be changed often and, therefore, very little of the operator's time is required for this operation. The still-pot temperature is measured, since it gives an early indication of a cut point and warns of the need for increasing the heat input to the still pot so as to maintain the boilup rate constant.

In starting the column the sample is pipetted or weighed into the still pot. Boiling chips are added. A hydrocarbon-insoluble grease or any other suitable grease is applied to the ground joint at the base of the column and the still pot is put in place and held with small springs hooked to the glass projections. A thermocouple is inserted into the thermowell of the still pot. The heater flask is raised around the still pot and insulated at the top by use of glass wool. The heater element within the heater flask is raised to within 12 mm. of the still pot. The heat is turned on and adjusted so that a boilup rate of approximately 80 drops per minute into the still pot is obtained within a half hour. After refluxing at this rate for one hour, the heat input is adjusted to give the desired boilup rate which is maintained constant. Except for the lower boilup rates, 3 to 4 hours then are usually required to reach equilibrium. After equilibrium is reached the electric timer, which is used in conjunction with a solenoid to operate the take-off valve, is adjusted and started to give the desired take-off rate.

Heat input to the column is controlled through a constant-voltage regulator, a toy transformer, and a Variac in series. Use of the constant-voltage regulator is recommended, since small

fluctuations in the line voltage materially affect the boilup rate in the column. The toy transformer (15 volts) and the Variac permit close control of the heat input.

The temperature readings which are obtained by use of the thermocouple in the head agree within 0.5° C. of the true boiling point of the overhead product, except during the distillation of the last portion of a constant-boiling fraction. During the distillation of the last portion of a constant-boiling fraction, the temperature increases gradually until it reaches the boiling point of the next higher constant-boiling fraction. The latter point has been found to agree well with the cut point expected when fractionating a known mixture of pure components which boil 6° C. or more apart. This behavior of the temperature is due to the position of the thermocouple which results in a temperature reading of the reflux to the column rather than of the vapor collected as overhead product. It is necessary, however, to have the thermocouple in its designated position rather than higher in the column in order to get constant-temperature readings and thereby follow the progress of the fractionation.

The operating holdup of the column is estimated to be of the order of 1.0 to 1.5 ml.

EFFICIENCY TESTS

Efficiency tests of this column were made under conditions of total reflux, using mixtures composed of carefully purified *n*-heptane and methyl cyclohexane. The purification procedure consisted of separately fractionating *n*-heptane (C.P. grade from California Chemical Corp.) and methyl cyclohexane (white label grade from Eastman Kodak Co.) in a column under conditions equivalent to a theoretical platage of about 30 to obtain constant-boiling center cuts. The physical properties obtained on these cuts are given below.

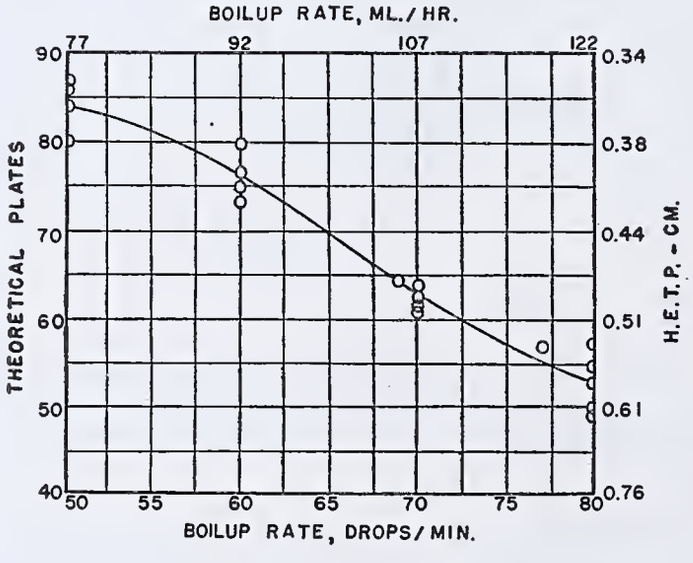

Figure 2. Efficiency Tests

Analyses of the *n*-heptane–methyl cyclohexane mixtures were made using the refractive index *vs.* mole fraction *n*-heptane data of Bromiley and Quiggle (*2*). Analyses of the distillate samples were obtained directly. Analyses of the still-pot mixtures which ranged from 20 to 40 ml. in volume were obtained by back-calculating from the analyses and volumes of the charge to the still pot and of the distillate samples, assuming no holdup in the column. The charge to the still pot was analyzed, measured, and changed periodically to make certain that cumulative errors did not invalidate the efficiency values. This method gave slightly lower efficiency values than would be obtained by direct analyses of the still-pot mixtures but was more convenient. Fenske's (*4*) formula (relative volatility = 1.07) was used for calculating the efficiencies in terms of theoretical plates.

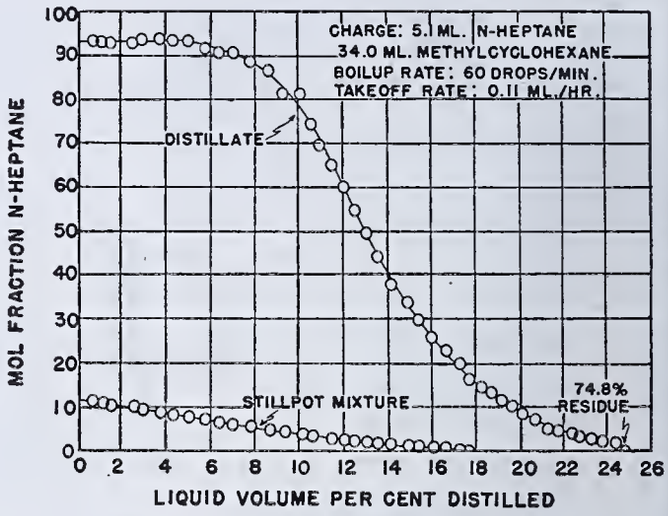

Figure 3. Distillation of Known Mixture

Table I. Efficiency Tests

Boilup Rate		Refractive Index of	Mole Fraction *n*-Heptane		Theoretical Plates	H.E.T.P.,
Drops/min.	Ml./hr.	Distillate	Distillate	Pot		Cm.
50	77	1.3888	0.970	0.084	86.5	0.35
50	77	1.3890	0.963	0.072	86.0	0.35
50	77	1.3890	0.963	0.080	84.0	0.36
50	77	1.3892	0.957	0.089	80.0	0.38
60	92	1.3888	0.970	0.121	80.0	0.38
60	92	1.3887	0.972	0.165	76.5	0.40
60	92	1.3892	0.957	0.118	75.5	0.40
60	92	1.3894	0.950	0.115	73.5	0.42
69	106	1.3916	0.878	0.083	64.5	0.47
70	107	1.3899	0.934	0.159	63.5	0.48
70	107	1.3900	0.930	0.161	62.5	0.49
70	107	1.3916	0.878	0.099	61.5	0.49
70	107	1.3917	0.875	0.097	61.5	0.50
77	118	1.3932	0.827	0.091	57.0	0.54
80	122	1.3928	0.840	0.098	57.0	0.54
80	122	1.3938	0.807	0.096	54.5	0.56
80	122	1.3961	0.730	0.069	53.0	0.58
80	122	1.3979	0.675	0.065	50.0	0.60
80	122	1.3928	0.840	0.154	49.5	0.62

Results of the efficiency tests are shown in Figure 2 and Table I. Very little or no liquid condensed in the head at boilup rates below 50 drops per minute.

	Boiling Point at Pressure of 750 Mm. Hg, ° C.	Refractive Index at 20° C.	
		Observed	Literature (*3*)
n-Heptane	97.8	1.3878	1.3877
Methyl cyclohexane	100.2	1.4233	1.4230

DISTILLATION OF KNOWN MIXTURE

The distillation of a known mixture of *n*-heptane and methyl cyclohexane was carried out at a boilup rate of 60 drops per minute into the still pot with a continual take-off rate averaging 0.11 ml. per hour. Samples of the distillate were taken every 2 hours and were analyzed by the refractive index data of Bromiley and Quiggle (*2*). Analyses of the still-pot mixtures were made by back-calculating as previously described. Results of this distillation are shown in Figure 3.

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NOTES ON ANALYTICAL PROCEDURES

Mercury Cleaning Apparatus for Continuous Operation

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THE present contribution is concerned with the mechanical means of bringing about thorough agitation and fine division of mercury in contact with any chosen cleaning solutions rather than with the chemical process itself. The apparatus consists of as many units, arranged in series, as are needed to accommodate the desired number of reagents and washes. A single unit is shown in Figure 1.

Each unit is prepared for operation in the following manner. In order to prevent escape of solution, a small amount of mercury is introduced into the outlet trap through the outlet by means of a funnel and short piece of rubber tubing, and a few drops are also placed in the inlet cup and trap. The solution reservoir is filled to within about 100 ml. of its capacity with the desired cleaning solution and connected with the unit by rubber tubing as shown. The air inlet is connected by pressure tubing to a source of air under pressure. Cleaning solution is poured in through the splash trap until the level reaches about halfway between bottom and top of the pump tube. The connections to the solution reservoir are freed of air by pinching the rubber tubing. Air under pressure is admitted cautiously through the air inlet until it is bubbling freely from the top of the ascending tube at *J* in the solution chamber. Mercury is then allowed to flow into the inlet cup of the first unit, and the rate of flow is adjusted to the desired rate of cleaning.

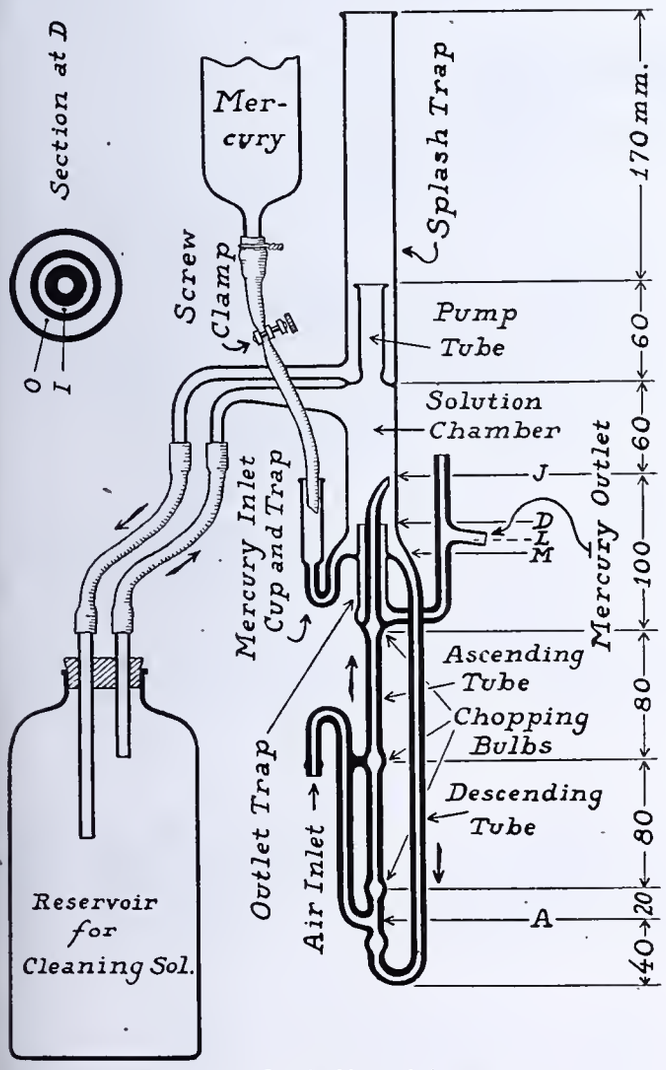

Figure 1. Single Unit of Apparatus

Splash trap and solution chamber	25–28 mm.*
Outlet trap (part surrounding ascending tube)	15–16 mm.*
Ascending and descending tubes	9 ± 0.5 mm.*
Inside diameters (bore)	3–3.5 mm.
Pump tube	15 mm.
Vertical distance, *D* to *L*	11–13 mm.
Vertical distance, *D* to *M*	20–22 mm.
Inside diameter of chopping bulbs	9–11 mm.

Dimensions are outside diameters except as noted. * Calculated for desired ratio of delivery to recirculation.

Air entering the ascending tube at *A* makes the column of fluid above this point lighter than in the descending tube and produces a rapid flow in the direction indicated by the arrows. Mercury entering the solution chamber through the inlet trap flows into the descending tube at *M* in large drops interspersed with solution from the chamber and thence down this tube to the ascending tube. The bulb below *A* retains enough mercury to act as a valve, retarding backward flow and favoring normal flow. Above *A*, the mercury is broken into smaller droplets by the air. The chopping bulbs in the ascending tube promote division, coalescence, and redivision of the mercury, thus frequently renewing the surface exposed to action of the solution and greatly extending its area. During their progress upward in this tube, these fine droplets are falling in relation to, and through, the more rapidly moving solution and air. Air, solution, and mercury are ejected into the solution chamber from the jet at *J*. The air passes out through the pump tube, carrying solution over with it and maintaining circulation of the solution between reservoir and solution chamber. The mercury impinges on the wall of the chamber and falls back in a shower of droplets to the dividing point, *D*, where a small part enters the top of the outlet trap. The larger portion falls to the bottom of the chamber and reenters the cleaning cycle.

The ratio of mercury delivered from the outlet to that recirculated is approximately proportional to the areas of the annular space between the outside of the ascending tube and the top of the outlet trap and that between the top of the trap and the wall of the solution chamber, indicated by *I* and *O*, respectively, in the insert. The ratio is fixed in the construction of each unit. A ratio of approximately 1 to 5 has been used.

Adjustments during operation are not critical within a wide range. Normal function is maintained by rates of flow of air from that of a "lazy" stream of bubbles to that which produces vigorous action and maximum turbulence. The limiting factor is back-pressure in the ascending tube. The rate of flow of mercury may be from the minimum obtainable to as much as 8 kg. per hour attained in one unit tested. Because of this wide range in rate of performance, each unit of the series will accommodate varying amounts delivered from the preceding unit, and no elaborate balancing of adjustments is required.

Air under pressure may be obtained from the usual laboratory system or from a pump delivering at a pressure of 700 grams per sq. cm. (10 pounds per square inch). Each unit requires a separate valve in a line carrying full pressure, so that the unit having lowest back-pressure cannot steal air from the others. In order to use one pump or one outlet of the laboratory air supply for several units, a metal manifold having a sufficient number of metal valves is suggested. If this is made of large pipe capped at one end, it can be filled with cotton or other material to serve as a filter to remove oil and dirt from the air.

To prevent escape of cleaning solution when a unit is not in use, the pressure tubing may be disconnected at the metal valve and the free end raised and hung on the supporting frame by means of a pinchclamp.

Batch operation of single units of a similar design, using successive changes of cleaning solution, has been tested over a period of two years at Wright Field, Dayton, Ohio, and at Bushnell General Hospital, Brigham, Utah, and proved convenient and satisfactory for processing very small amounts of mercury. A single unit of the present apparatus may be used similarly when economy of space is more important than the advantages of simultaneous multiple operations.

The advantages of the apparatus are fine division of mercury and prolonged contact with the solution, elimination of fine orifices which tend to become clogged with dirt, automatic operation requiring little attention, relatively small size, light weight, adaptability to few or many simultaneous cleaning operations and to very small and very large capacity requirements, and small holdup.

Cleaning solutions that have been used satisfactorily in this type of apparatus are 10% lactic acid, 5% sodium hydroxide, 5% sulfuric acid, 3 to 10% nitric acid, and water, usually in the order named when more than one is used. Control of operations has been based on such criteria as speed of coalescence of droplets, freedom from blemish of the mercury surface, freedom from stain on glass surfaces in prolonged contact with the mercury, and clean appearance during distillation. More specific data have not been sought. It has been assumed that quality of product is inversely related to its rate of flow.

Figure 1 is drawn to scale, except that some diameters are slightly exaggerated for printing. Operable models can be made within a wide range of dimensions and proportions.

Ascending tubes have been used having inside diameters up to 4 mm. and lengths up to 600 mm. The descending tube should have the same inside diameter as that of the ascending tube; greater bore results in unstable operation, and smaller bore yields little if any advantage. The bore of the jet should not be tapered to less than half that of the rest of the ascending tube; if too small, the back pressure is excessive. The ascending and descending tubes should be built as close to each other as possible to avoid collection of excessive amount of mercury in the horizontal part of the loop. A slight funnel-like expansion of the top of the descending tube favors normal flow. A slight constriction at the bottom of the pump tube is intended to improve pumping action.

The side tubes for connection with the solution reservoir are separated horizontally sufficiently to avoid difficult glass blowing and excessive fragility. The one carrying the return to the solution chamber is slightly funnel-shaped and inclined to provide for return of mercury droplets to the chamber. The mercury inlet cup and trap and the mercury outlet are actually in a plane perpendicular to that of the ascending and descending tubes and air inlet. The length of the splash trap may be increased for alkaline or soapy solutions, or caprylic alcohol may be used to break any foam. Any convenient bottle is suitable for the solution reservoir.

Units of this apparatus may be obtained from Harshaw Scientific Company, Cincinnati, Ohio.

Assay of Hydroquinone

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A COMPARISON has been made of the iodometric and cerometric titrations in the assay of hydroquinone.

IODOMETRIC METHOD. In this method an excess of standard iodine solution is added to a solution of the hydroquinone and the excess iodine is back-titrated (*1*, *4*, *5*, *6*). The sources of error have been studied systematically with the following results:

1. In order to have the oxidation of hydroquinone go to completion the pH of the solution must be greater than 6. Many authors (*1*, *5*, *6*) recommend a solution of sodium hydrogen carbonate (pH 8.4) as a suitable reaction medium.
2. If the reaction is carried out in a hydrogen carbonate solution, the solution should be protected from air to prevent air oxidation of hydroquinone (*3*). The back-titration of excess iodine must be made with arsenious acid, not with sodium thiosulfate.
3. Another source of error, not discussed in the literature, is that iodine reacts to some extent with quinone to form an orange or brown colored product. From a number of experiments it was concluded that the back-titration of iodine must be made relatively quickly in order to reduce the extent of reaction of iodine with quinone to a minimum. The orange or brown compound formed also obscures the end point.

The following procedure, taken from the Laboratory Manual of the Rubber Reserve Company and modified slightly, was found to give results almost identical with the procedure recommended by Kolthoff (*4*).

Procedure. Accurately weigh a sample of 0.15 to 0.2 gram and transfer it to an Erlenmeyer flask. Add 25 ml. of water, dissolve the hydroquinone, and add 5 grams of solid carbon dioxide and 2 grams of sodium hydrogen carbonate. Cover the flask with a small watch glass and swirl the flask intermittently until the dry ice has disappeared. Then warm the solution to room temperature but not above. Do not allow the sample to stand any length of time at this point. Add 1 ml. of starch indicator and titrate immediately with 0.1 *N* iodine solution to the starch-iodine end point. Add just 1 ml. of iodine solution in excess and back-titrate with 0.05 *N* arsenious acid solution to a light yellow color.

The error in both Kolthoff's procedure and that given above was found to be +0.3%, and the precision is of the same order of magnitude.

CEROMETRIC METHOD. Titration of hydroquinone with ceric sulfate solution was recommended by Furman and Wallace (*2*), who found that the end point could be detected potentiometrically or with diphenylamine or diphenylamine sulfonate indicators. The authors have found that ferrous phenanthroline is a very suitable indicator.

The average error of ten titrations with ceric sulfate (0.1 *N*) was 0.02%.

In the titration of three commercial samples of hydroquinone of 96 to 100% purity, iodometric and cerometric methods gave the same results to within ±0.3%.

The cerometric method is recommended for the assay, routine or otherwise, of hydroquinone, except when the sample contains impurities, such as oxalate, which are oxidized by ceric sulfate but not by iodine.

SUMMARY

The ceric sulfate procedure for the titration of hydroquinone is accurate and precise to 0.02%. Ferrous phenanthroline can be used as an indicator. Under the most favorable conditions the iodometric method gives an error of +0.3%, due to reaction of quinone with iodine.

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Constant-Level Feeder for Thermostatic Baths and Continuous Evaporators

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VARIOUS forms of useful constant-level devices for thermostatic baths at elevated temperatures have been described *2–14, 16–23*). Such devices may be broadly classified into three roups: (1) The common form of constant-level attachment ot employing the siphon principle, but working in combination ith an overflow arrangement. To attach this form to the bath, is necessary to drill a hole in the bath. (2) Devices depending pon the siphon principle in combination with an overflow arngement, requiring no drilling of hole to attach to the bath. 3) Devices using siphon system in accordance with the prinple of the Mariotte bottle, eliminating drilling of any hole in the ath vessel.

Objections to the first type have been pointed out by Wilde *20*). Devices of the second type have certain advantages, but quire a thermostat in a place easily accessible to a water tap nd drain. They waste water, sometimes even hot water, and r this reason, they consume more of the gas or electricity used r heating in conjunction with thermoregulators. If the tap ater is not soft enough, these devices cannot be run with disled water to avoid scale formation in the bath. Some of these evices involve a lot of glass blowing. In many cases, it is necesary to pay periodic attention to adjustment of water flow.

The third type eliminates the defects of the first two types; owever, reservoirs must be frequently refilled, though attempts ave been made by some authors to minimize refilling. The resent paper deals with the third type, simplifies the construcon of older forms, indicates improvements over previous forms, nd discusses both advantages and disadvantages.

The device is shown in Figure 1. Clamps and other supports re not shown in the diagram.

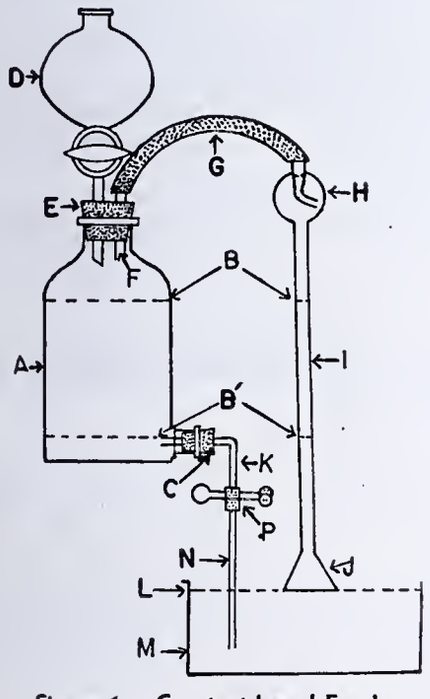

Figure 1. Constant-Level Feeder

A. Aspirator bottle, of any convenient size. *B*. Water level at beginning. *B′*. Water level at end. *C*. Single-holed rubber stopper to fit bottom tubulature outlet of *A*. *D*. Separatory funnel of any size. *E*. Double-holed rubber stopper to fit mouth of *A*. *F*. Connecting glass tube, 6-mm. diameter. *G*. Rubber tube. *H*. Splash head such as that of Kjeldahl's apparatus. *I*. Long stem of funnel *J* fused or connected by short rubber tube to *H*. Rim diameter of *J* 5 cm., diameter of *I* 6 mm. *K*. Delivery tube, 3-mm. diameter. *L*. Actual level of water in thermostatic bath *M*, coinciding with rim of *J*. *N*. Feed tube of glass, 3-mm. diameter. *P*. Pinchcock and short rubber tube connecting *N* and *K*.

A serves as the water reservoir, *D* being the refiller. *N* is the feeder to the bath. *G*, being flexible, permits adjustment of level *L* in *M*. The splash head, *H*, prevents carrying over water from *M* back into *A*. The limb, *HIJ*, can be regarded as a balancer of pressure in *A* and consequently as a level controller in *M*. Funnel *J* is necessary, as otherwise, even if the level falls below *L*, a column of water remains suspended in *I*, owing to surface tension effects, and will throw the feeder out of action.

To start working, the lower end of *N* is immersed well below level *L* which, in turn, is adjusted to a predetermined height by the position of the rim of *J*. *P* is closed and *A* is filled with water through *D*. While filling *A*, *P* should be closed, as otherwise the water level in *M* rises above *L* by draining through *N*. Extra pressure produced by compression of air enclosed in the space above the water level in *A* during filling through *D* will be relieved by the escape of air bubbles through *F* and ultimately through *J*. If filling of *A* is stopped by closing the tap of *D* and if *P* is then opened, a slight readjustment of the levels of water in both *A* and *M* takes place and water rises in tube *I* until it is the same as in *A* as shown at *B*. If the water is cold at first, the level in *M* stands a fraction of a centimeter above *L*. As heating and evaporation progress, the level falls to *L* and remains practically constant throughout. Whenever the level falls very slightly below *L* owing to continued evaporation, the water seal at the rim of *J* is broken and air bubbles escape into *A* through *HIJ*. The pressure of air enclosed above water in *A* thus increases slightly, thereby driving more water through feeder *N* until the level in *M* rises again to *L* and reaches the mouth of *J*. This process continues as long as there is a water head available in *A*. When the water becomes hot, the water in the balancer may rise a little higher than in *A*, owing to diminution in density of water at elevated temperatures.

Operation is an application of the principle of the Mariotte bottle. The flow of water from *A* depends jointly upon the head of water and the pressure of air enclosed in *A*. Since the pressure of air in *A* is controlled by the balancer limb, *HIJ*, it is evident why the flow of water from *A* into *M* should stop when the mouth of *J* is sealed by water reaching level *L*.

The above arrangement is a modification of Gerdel's device (*7*), which has the following defects: To refill, the stopper of the reservoir bottle must be removed or a separatory funnel attached through the stopper by boring a fourth hole in it; this leads to an undesirable state of congestion. Siphoning continues during refilling if done through a separatory funnel, and thus raises the level of water in the bath. At the boiling temperature, some water is carried from the bath back into the reservoir, owing to the increased frequency of air bubbles rising in the balancer limb. This may not be a serious defect so far as the thermostatic bath is concerned, but if all the distilled water in the reservoir is not used up by the bath, the water remaining will be rendered unfit for other purposes, owing to contamination with the water in the bath. At elevated temperatures, air dissolved in water is expelled and air bubbles accumulate at the head of the siphon, hindering or stopping the siphoning operation. These defects are eliminated in the improved device here described.

The advantages of this arrangement are obvious. It can be made of materials readily available in a good laboratory. It feeds water to the bath only when necessary, eliminating wastage of hot water. It can be set up in any desired place. It avoids any damage to vessels, especially those of glass or stoneware. It can be worked even at the boiling temperature. Any desired water level in the bath can be obtained quickly by adjusting the position of the rim of *J*. The device and the bath are entirely independent of each other, hence substitution of either is possible. As there is no continuous flow of water, adjustment of flow needs no attention. Dissolved air expelled from hot water in the bath has no place to accumulate to obstruct siphoning operation, as the flow of water depends mainly upon the hydrostatic head. There is no complicated glass blowing, as the equipment needed is of standard type. It can be used with any quality of water, being specially suitable for distilled water. Refilling of the aspirator

bottle is necessary; but this is not serious if an aspirator bottle of large capacity is chosen. For a thermostatic bath of normal size (*13*), refilling may be necessary once a day. If a metal tank reservoir is available, so much the better.

CONTINUOUS EVAPORATORS. Evaporators may be of various types (*1*), but they are either open pans or closed bodies employing vacuum. They may be of the small-scale laboratory size

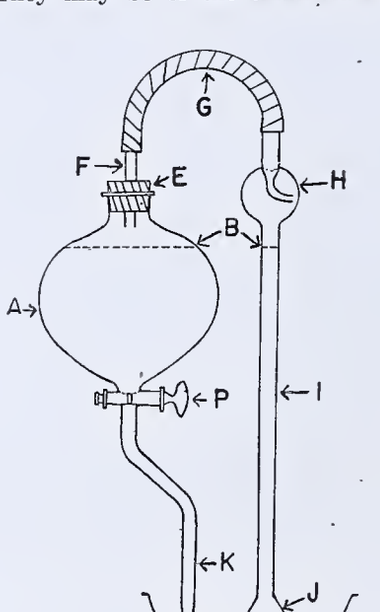

Figure 2. Feeder for Continuous Evaporator

or the larger size used in industry. The essential features of a laboratory-size continuous open evaporator are described in the present paper; results referring to a vacuum evaporator will form the subject matter of a future paper.

Literature on the subject of constant-level feeders for continuous evaporators, particularly laboratory or pilot-plant size, is rather scanty. A device for maintaining a constant level during continuous evaporation in the determination of total dissolved solids in water recently described (*15*) has to be specially made to given specifications, and so does not permit the choice of materials ordinarily available in a laboratory.

If an evaporating basin is substituted for a thermostatic bath, the arrangement in Figure 2 can be readily understood. If a separatory funnel is substituted for an aspirator bottle, the liquid to be evaporated can be completely drained, especially when a measured volume of liquid is placed in it. In order to accommodate the arrangement properly, the stem of the separatory funnel is bent as shown, and drawn out to a narrow tip of about 3-mm. diameter. Air bubbles that may remain in the stem of the separatory funnel may be expelled by pinching a rubber tube temporarily attached at the tip.

If a rubber connection is undesirable, *H* and *I* may be joined by fusion, as the liquid splashes right up to the splash head, *H*. The apparatus is simple and easily constructed and can give satisfactory service even in analytical work.

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Adsorption Apparatus without Stopcocks for Use with Hydrocarbon Gases

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THE development of the Brunauer, Emmett, and Teller low-temperature adsorption isotherm method for determining the surface area of finely divided substances (*2–7*) has led to widespread acceptance and use of this procedure in many laboratories. The validity of the method has been further established by the work of Harkins and Jura (*8, 9, 10*), whose approach to the problem involves precision calorimetry and makes no assumption as to the effective cross-sectional area of the adsorbate. Their measurements yield surface area values in excellent agreement with those of the Brunauer-Emmett-Teller method.

APPARATUS FOR INERT GASES

The Brunauer-Emmett-Teller method has been used in the author's laboratory for the past five years for determining the surface area of a variety of fine pigments. During this period, an apparatus which is accurate and reasonably foolproof in operation has been developed. Figure 1 shows the apparatus employed for surface area measurements using nitrogen as the adsorbate.

The apparatus, as originally constructed, was similar to that used by Benton and White (*1*). It consists of a constant-volume buret with two internal blue glass pointers sealed in the constant-volume arm at suitable points to provide two volume figures, one approximately twice the other, for versatility in operation. Conventional mercury reservoirs are used to control the mercury levels in buret *J* and manometer *I*.

The usual 120° three-way stopcock first used on the gas buret was unsatisfactory. This particular design of stopcock has such a short distance between each two of the three side arms that it frequently leaks under high vacuum conditions. To overcome this difficulty, stopcock *D* (fabricated by Eck and Krebs, New York, N. Y., shown in detail in Figure 2) was employed. This cock has very little dead space in the bore, yet has a full 180° between the two side arms. Figure 2 also shows a special adsorption cell, *C*, designed to reduce operating time when a series of samples of the same density is to be measured. Since the dead space in this cell remains constant as long as samples of the same

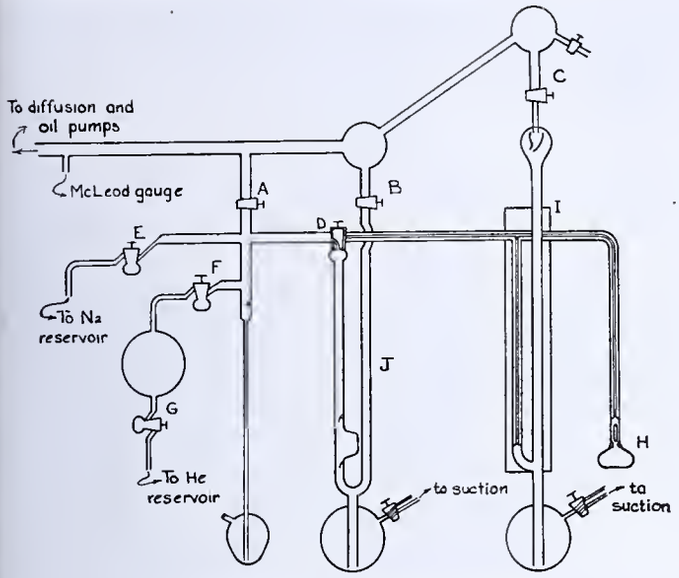

Figure 1. Apparatus for Inert Gases

veight and density are used, a series of measurements may be nade under these conditions with a single helium dead space calibration. A considerable gain in time is thus effected over the conventional "seal on and off" type of cell. The ground joint is at least 5 to 7.5 cm. (2 to 3 inches) from the surface of the liquid nitrogen bath; however, conduction by the glass necessitates a water jacket on the female member of the joint to prevent the grease from freezing. The long capillary tube on the lower part of the male joint member effectively reduces dead space to a minimum.

APPARATUS FOR HYDROCARBONS

Some months ago, it was decided to extend adsorption studies to a number of hydrocarbon gases. Because of the effect of these gases on stopcock grease, an apparatus with no stopcocks in contact with the adsorbate gas was indicated. Since apparatus of this type described in the literature left much to be desired for the author's purposes, the apparatus pictured in Figure 3 was finally designed and constructed. Considerable experience has been gained with this apparatus in conjunction with a study of the heats of adsorption of various gases on fine pigments. The results will be discussed in a forthcoming paper.

The apparatus, aside from the usual mercury cutoffs operated by reservoirs K, is essentially similar to that shown in Figure 1 except for the unique controls, A and B. These controls (shown in detail in Figure 2) were developed to solve the problem of confining a volume of gas in buret J and varying its volume with the buret mercury level independent of the levels in the inlet and outlet tubes. By making use of Pyrex hypodermic syringes, b, and ground-glass valves, a, positive control of the gas is obtained with no contact of the gas with grease. (Medical Center brand syringes were used, manufactured by Becton, Dickinson and Co., Rutherford, N. J.) In each case, the piston of the hypodermic syringe is connected to the ground-glass valve by means of glass toggle joints, c, which permit enough freedom of movement in the male sections of the ground-glass valves so that the valve can be tightly seated in the female section. The syringes proper are greased, but since mercury from the reservoir always covers the toggle-joint connection, no contact between gas and grease is permitted. Thus, the valves connected to the syringe pistons may be operated manually, preferably by thumbscrews of proper design. The valve in the left-hand arm of unit B is of the simple floating type.

Operation of these units is simple and positive. With all mercury levels lowered, the entire apparatus is evacuated to the desired working pressure. Buret J is then filled with gas as follows: The right-hand valve of B being closed by means of the thumbscrew, the mercury control cock of B reservoir is opened to the atmosphere. This places a pressure of about 660 mm. on both valves. After the mercury level in the buret is raised to about the center of the U-tube, J, and the mercury in A to slightly above the higher toggle joint, the desired quantity of gas may be taken into the buret from either the helium or hydrocarbon reservoir. The mercury level in unit A is then raised to point L, and both valves are closed tightly by means of the thumbscrews. Now the pressure on the gas in the buret may be varied at will (between 0 and about 550 mm.) with no resultant change in the mercury levels of A and B. Hence the mercury level in buret J may be adjusted to touch the desired pointer and the pressure read off the right arm of the buret which is calibrated in millimeters.

With the buret filled, the transfer of known amounts of gas from buret to adsorption cell involves manipulation of unit B only. After raising the mercury level in manometer I to shut the cell unit off from the line, the control stopcock on the reservoir of B is cautiously cracked to suction until the mercury level in B proper falls to slightly below the bottom of the floating valve in the left arm. This valve is now open but the right-hand valve is still closed by the pressure maintained on it by the thumbscrew. Manipulation of the thumbscrew will now admit suitable amounts of gas to the adsorption cell. To close control B in order to measure the volume of the gas remaining in the buret, the control

Table I. Reproducibility of Results

Pressure Difference (Buret) *Mm.*	Pressure Reading (Cell) *Mm.*	Calculated Cell Volume *Ml.*	
T = 22.8° C.			
70	96.8	26.71	
152	211.1	26.60	Cell I
229.5	318.3	26.64	
T = 23.3° C.			
64.3	121.3	19.51	
112.0	212.0	19.48	Cell II
169.3	320.3	19.48	

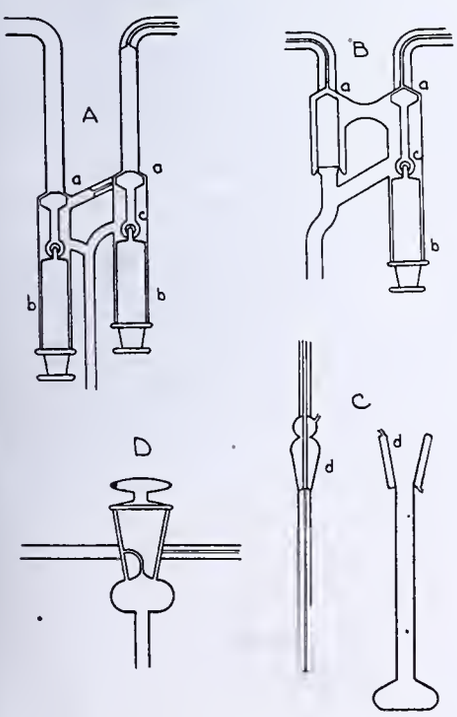

Figure 2. Detail of Stopcock

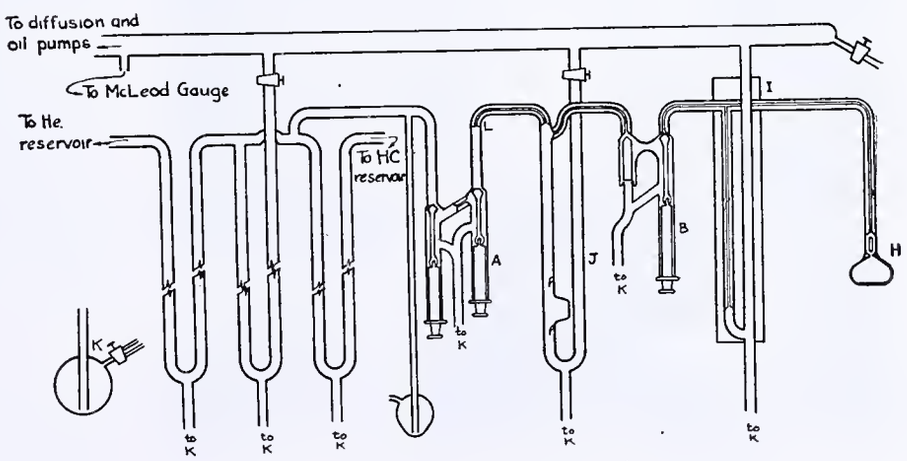

Figure 3. Apparatus for Hydrocarbons

cock of B reservoir is again opened to the atmosphere, the rising mercury forcing all of the gas trapped in B either into the buret or into the adsorption cell where it will be measured in either case.

The reproducibility of results obtained with this apparatus has been found to be fully equal to that of the apparatus shown in Figure 1, and is well within 1%, as illustrated by the data in Table I, which were taken during the calibration of the apparatus, using two cells of different size. The agreement of the data shows clearly how accurately the transfer of small amounts of gas may be accomplished with this apparatus.

ACKNOWLEDGMENT

The writer wishes to express his appreciation to Ralph A. Beebe and Johnathan Biscoe for their helpful suggestions concerning the development of this apparatus.

SUMMARY

A high-vacuum apparatus without stopcocks has been designed which permits efficient handling of hydrocarbon gases in adsorption work. Positive control of the gases has been achieved through "mercury stopcocks" which involve simply hypodermic syringes and ground-glass valves.

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Rapid Qualitative and Quantitative Determination of Barbiturates from Postmortem Specimens

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A METHOD is described for the separation of barbiturates from postmortem specimens by extraction with aqueous sodium hydroxide. Proteins are coagulated with sodium tungstate upon acidification with sulfuric acid and, following filtration, the filtrate is extracted with ether. Evaporation yields the barbiturates usually in crystalline form ready for qualitative and quantitative determination. The yield is complete. The elapsed time required is 90 minutes.

The prevalent use of barbiturate drugs has resulted in a steady increase in the number of postmortem cases in which barbiturate is the issue. The introduction in this laboratory of the method described below has resulted in large savings in time and reagents.

PROCEDURE FOR EXTRACTION OF BARBITURATE FROM BLOOD AND VISCERA

1. Measure 60 ml. of blood (or minced viscera), 410 ml. of distilled water, and 10 ml. of 10% sodium hydroxide into a 1-liter flask. Shake 5 minutes.
2. Add 60 ml. of 10% sodium tungstate. Add slowly 60 ml. of 0.67 N sulfuric acid with continuous shaking, and acidify with 18 N sulfuric acid until acid by Universal indicator paper.
3. Filter to collect 450 ml., extract with an equal volume of redistilled ethyl ether, and shake 5 minutes. Separate from the water phase and evaporate.

The relatively large volume of ether prevents emulsions, which occasionally occur with smaller quantities of ether. The evaporation is normally performed in distillation equipment. The ether is recovered and re-used in later extractions. This procedure permits the attainment of lower blanks.

4. Weigh, deduct blank, and calculate milligrams per 100 grams.

Determination of Blanks. Add 250 mg. of phenobarbital to 60 grams of liver and extract according to preceding directions. The average recovery is 253 mg., representing the barbiturate plus blank. Extract in the same way 60 grams of liver. The average residue is 3 mg.

The established average of foreign matter per 60 grams of liver may be subtracted in the quantitative determination of high positive cases. Low positive cases call for a colorimetric quantitative determination of barbiturate.

5. Identify the presence of barbiturate by the customary Zwikker-Koppanyi method.

The Rotondaro (*1*) method may be employed for final purification of the residue, in which case the color effects with the Zwikker-Koppanyi reagents will be brighter. This is not necessary, however, as the presence of a few milligrams of barbiturate may be established conclusively without Rotondaro purification.

DISCUSSION

The distinctive feature of this method is the extraction of a small quantity of tissue with sodium hydroxide which brings the barbiturate directly into solution, separating it simultaneously from fats and oils. The coagulation of proteins yields a clear solution, substantially purified, so that the final ether extraction is rapid and complete. The final residue is usually crystalline, ready for qualitative and quantitative determination. Melting points can also be established after sublimation. Secanol residues and, sometimes, nembutal are noncrystalline, but the latter also becomes crystalline after evaporation of added water. In practice, the following residual quantities of ingested barbiturates are encountered: for secanol, up to 3 mg. per 100 grams of liver; for nembutal (pentobarbital), up to 16 mg., and for phenobarbital, up to 39 mg.

Cases are very rare where barbiturate is accompanied by other substances extractable by ether from an acid medium (such as salicylic acid, sedormid, or acetophenetedin). Since therefore any abnormal weight exceeding 40 mg. of ingested barbiturate per 100 grams of tissue would attract the attention of the toxicologist, it could not be overlooked, and would indicate interference of the above type. This makes the method practically specific for commonly used barbiturates.

The advantages of the method become obvious if it be contrasted to the customary method employing ethyl alcohol. Primarily, in order to ensure recovery from low positive barbiturate cases, large quantities of tissue, up to 400 grams, are subjected to four alcohol extractions, followed by evaporation. This procedure, besides being cumbersome, yields a gummy paste, formed by heating the fatty and oily components of human tissue which are extracted with the barbiturate. Subsequent water and ether extractions yield a final noncrystalline residue requiring additional purification. The elapsed time required is from 2.5 to 3 days. Endeavors to reduce the time by working with smaller amounts of tissue and decreasing the number of consecutive alcohol extractions often leads to failure in low positive cases.

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Micro-Kjeldahl Determination of Nitrogen in Gramicidin and Tryptophan

Comparison of Gunning-Arnold-Dyer and Friedrich Methods

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WORK at this laboratory on the modification of gramicidin through reaction with formaldehyde (*6*) required a simple nd reliable micromethod for the determination of nitrogen in his antibiotic and in certain of its derivatives and components.

Hotchkiss and Dubos (*3*) reported that the Dumas combustion ethod was unreliable with gramicidin and that consistent results ere obtainable with the Kjeldahl method only when the sample vas given a Friedrich pretreatment with hydriodic acid prior to he usual digestion (*9*). Other workers (*2*, *11*) have also used the ydriodic acid reduction method for the determination of nitro- en in gramicidin.

The need for the hydriodic acid pretreatment was attributed *3*) to the failure of the Kjeldahl method to show more than 88 to 5% of the total nitrogen of pure tryptophan, which constitutes p to 40% of the gramicidin molecule. Van Slyke, Hiller, and illon (*12*) reported that, with pure tryptophan, the Kjeldahl nethod gave variable results averaging about 90% of the theo- etical. Since the experimental work for the present paper was ompleted, Miller and Houghton (*8*) have reported 92.8% recov- ry of nitrogen from tryptophan solutions with a digestion time of hours and 97.7 to 100.7% recovery for a 7-hour digestion. The resence of half of the nitrogen of tryptophan in the indole ring nay explain the difficulty experienced in obtaining a quantita- ive yield of nitrogen from this compound.

Experience with the Gunning-Arnold-Dyer method as de- cribed by Clark (*1*) led the authors to believe that the nitrogen f tryptophan could be completely recovered by this method with- ut recourse to the time-consuming and tedious Friedrich hydri- dic acid pretreatment. This paper reports a comparison of the itrogen contents of several samples of gramicidin and *l*-trypto- han and their derivatives as determined by the two methods.

METHODS

A 5- to 7-mg. sample is weighed by difference into a 30-ml. Kjeldahl flask and digested in the presence of a 5- to 6-mm. glass bead with 1.5 ml. of concentrated sulfuric acid, 500 mg. of potassium sulfate, and 40 mg. of mercuric oxide. The digestion is carried out as described by Clark (*1*) for the Gunning-Arnold-Dyer method for a minimum of 1 hour or until the digest is clear and colorless for 0.5 hour. After cooling, a drop of ethyl alcohol is added and the mixture is again heated until it is colorless. The digests must be boiled vigorously during the later stages of the digestion; otherwise low results may be obtained. (With the samples described in this paper the total time of heating was usually 80 to 85 minutes.) Distillation of the sample is carried out according to Clark's method, but the ammonia is received in 5 ml. of 2% w/v boric acid containing 1% v/v of the mixed indicator of Ma and Zuazaga (*7*). The absorbed ammonia is titrated with 0.01 *N* hydrochloric acid in a final volume of 25 ± 2 ml. The acid is standardized by the determination of nitrogen in acetanilide (N.B.S. standard sample 141) and/or cystine (N.B.S. standard sample 143). Blank determinations are made daily and other usual precautions are taken.

The authors' modification of the Friedrich method varies from that described by Clark (*1*) in the same particulars as does their modification of the Gunning-Arnold-Dyer method and, in addition, they use red phosphorus with the hydriodic acid as prescribed by Pregl and Roth (*9*).

DISCUSSION OF RESULTS

l-Tryptophan from two sources was analyzed without purification; 3,4,5,6-tetrahydro-4-carboline-5-carboxylic acid was prepared from tryptophan and formaldehyde according to the directions of Jacobs and Craig (*4*). The remainder of the samples used in this study were kindly supplied by H. S. Olcott and co-workers of this laboratory, who will report on their preparation and significance elsewhere. All results have been corrected for moisture.

The percentages of nitrogen found by the Gunning-Arnold-Dyer method in a number of samples of gramicidin, tryptophan, and their derivatives are compared in Table I with those found by the Friedrich method. These data show that there was no significant difference between the results obtained by simple digestion and those obtained by longer pretreatment for any of the samples.

Table I. Nitrogen Analyses of Gramicidin and Tryptophan and Their Derivatives

Compound	Gunning-Arnold-Dyer Digestion, % *N*		Friedrich Digestion, % *N*	
l-Tryptophan (Merck), 13.5 to 13.8%	13.6	13.6	13.6	13.7
l-Tryptophan (Eastman 2025), theory 13.7%	13.5	13.6	13.8	13.8
N-acetyl tryptophan, theory 11.4%	11.3	11.4	11.3	11.4
N-benzoyl tryptophan, theory 9.09%	9.03	9.05	8.97	8.98
l-Tryptophan, HCHO-treated[a], theory 12.96%	12.9	12.9	12.8	12.8
Gramicidin (33% tryptophan, m.p. 208° C.)	14.2	14.3	14.2	14.2
Gramicidin (37.5% tryptophan, m.p. 225° C.)	14.5	14.5	14.4	14.5
	14.5	14.6	...	...
Gramicidin, HCHO-treated	13.4	13.4	13.2	13.4
Gramicidin, HCHO-treated, crystalline	13.3	13.4	13.3	13.3

[a] 3,4,5,6-Tetrahydro-4-carboline-5-carboxylic acid.

The recoveries of nitrogen by simple digestion from *l*-tryptophan and its acetyl and benzoyl derivatives were between 98.5 and 100%. Very slightly higher recoveries of nitrogen in tryptophan can be obtained if the digestion is prolonged 30 minutes over that prescribed by Clark. With the longer digestion, recoveries ranged from 99 to 100%. More prolonged digestion was without effect. It may therefore be assumed that the recovery is equally good for gramicidin, since *l*-tryptophan is the only component of this material that has been reported to give low nitrogen values.

Nitrogen in the carboline ring might be expected to be resistant to oxidation. However, analysis of 3,4,5,6-tetrahydro-4-carboline-5-carboxylic acid showed a nitrogen recovery of 99.5% by the simple digestion.

The exact nature of the product obtained on treatment of gramicidin with formaldehyde has not yet been established. However, it is doubtful that the nitrogen occurs in a linkage different from that present in other samples used in this study.

The complete recovery of nitrogen from the indole ring of tryptophan is in accord with the findings of Clark (*1*), Kaye and Weiner (*5*), and Shirley and Becker (*10*), who demonstrated the applicability of mercury as a catalyst to similar refractory nitrogen-containing rings, such as pyridine, quinoline, purine, pyrimidine, thiazole, and pyrole. Working with solid samples the authors have been unable to confirm the results of Miller and Houghton (*8*), who recovered only 92.8% of the nitrogen in tryptophan solutions after a 2-hour digestion with mercuric oxide. The authors found, for example, 92.7% recovery after 35 minutes of total digestion; 97% after 55 minutes; 99.4% after 85 minutes (regular digestion); and 100% after 115 minutes.

SUMMARY

Mercuric oxide has been shown to be a suitable catalyst for the microdetermination of nitrogen in tryptophan (indole and amino nitrogen) and its acetyl and benzoyl derivatives. By its use the recovery of nitrogen from the carboline ring is complete. With mercuric oxide as a catalyst, nitrogen may be determined in gramicidin and in the gramicidin-formaldehyde reaction product without the customary pretreatment with hydriodic acid.

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Method for Purifying Beta-Picoline and a Test for Purity

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AND
SIDNEY A. SAVITT AND DONALD F. OTHMER
Polytechnic Institute of Brooklyn, Brooklyn, N. Y.

BETA-PICOLINE, an important raw material for the manufacture of nicotinic acid, is not usually available in purity higher than 90 to 95%; and this is made from a commercial picoline fraction containing about 30% β-picoline. The boiling point range of the 90 to 95% material is 143–5° C., and the usual impurities consist of γ-picoline and 2,6-lutidine. Further fractional distillation does not increase the purity, since the boiling points of all fall in a very narrow range. The α-picoline and the other lutidines boil at temperatures sufficiently removed to allow separation by fractional distillation.

A suitable chemical method was desired which would allow quantitative separation of γ-picoline and 2,6-lutidine from β-picoline and qualitative demonstration of even very small traces of these impurities in a given sample of β-picoline.

γ-Picoline and 2,6-lutidine may be converted to pyrophthalones (*1*, *2*). β-Picoline does not react. For purifying 1000 grams of approximately 95% β-picoline, 250 grams of phthalic anhydride and 250 grams of acetic anhydride are boiled together under reflux for 4 hours. The mixture is cooled and poured into 5000 cc. of distilled water and then made strongly alkaline with sodium hydroxide solution (concentrated) added slowly. A dark brown precipitate of the pyrophthalones is filtered off. When the filtrate is distilled an azeotropic mixture of β-picoline and water comes over at 97° C. The distillate is dried with solid sodium hydroxide, which separates two layers: picoline above the aqueous sodium hydroxide. The latter is drawn off, and solid sodium hydroxide is added again, until no further separation into two layers is effected.

The picoline is separated from the solid sodium hydroxide and distilled, and the fraction distilling at 143–4° C. is collected. The entire procedure is repeated, starting with the addition of phthalic and acetic anhydrides, until the mixture of the impure β-picoline and the two anhydrides give no yellow color on heating. It is usually necessary to repeat this three times.

TEST FOR PURITY

One hundred grams of β-picoline, purified as above until no yellow coloration developed, were added to 25 grams of phthalic anhydride and 25 grams of acetic anhydride. Several such mixtures were boiled under reflux for 10 minutes after adding small amounts, down to 0.008 gram, of substantially pure 2,6-lutidine.

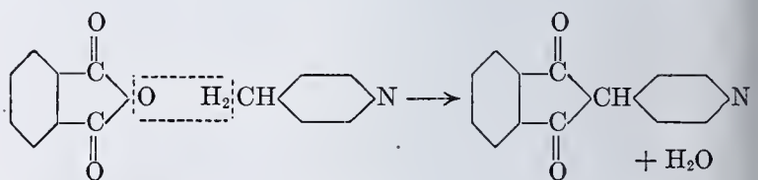
The yellow coloration developed in all cases, decreasing in intensity with smaller amounts of lutidine. The sample containing 0.008 gram of 2,6-lutidine was a pale yellow, showing that as little as 0.01% of this material could be detected.

The same results were obtained when the procedure was repeated using γ-picoline as the impurity.

These tests using varying amounts down to almost infinitesimal traces of 2,6-lutidine and γ-picoline, which are the most common impurities of β-picoline, show conclusively the extreme sensitivity of this method of purifying and testing β-picoline. Furthermore they show that β-picoline so purified is substantially free of the impurities normally associated with it.

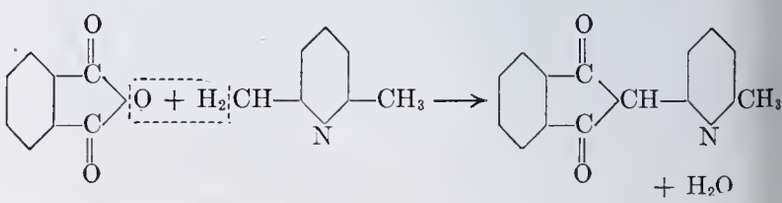
DISCUSSION

The phthalic anhydride reacts with the methyl groups of picolines when present in the 2 and 4 positions to give yellow pyrophthalones, but no reaction occurs with the picoline having a methyl group in the 3 position. The condensation takes place as follows:



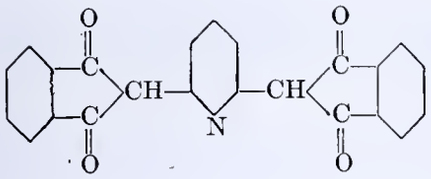
Phthalic anhydride γ-Picoline Pyrophthalone

With the 2,6-lutidine, the condensation reactions may be represented as follows:



Phthalic anhydride 2,6-Lutidine

With a large excess of phthalic anhydride and acetic anhydride, both methyl groups react as follows:



The pyrophthalone compounds are yellow dyes, the properties of which have been described by Hewitt (*1*).

This formation of pyrophthalones may be traced to the presence of 4-picoline or 2,6-lutidine, or both; and it has been recognized that a methyl group in position 2, 4, or 6 is necessary for condensation with carbonyl groups. Two hydrogen atoms from the picoline and one oxygen atom from the phthalic anhydride are released to form water.

However, the methyl group in the 3 position is entirely unreactive and therefore no color develops. The β-picoline may be considered absolutely free of its isomers and homologs when there is no appearance of color following this treatment.

Pure β-picoline is extremely hydroscopic and precautions should be taken to keep it absolutely dry.

Slight traces of any of the usual impurities in a sample of β-picoline will be detected by the appearance of yellow color on refluxing with phthalic anhydride and acetic anhydride. Therefore, if no color develops, the picoline may be considered free of these impurities. The sensitivity of the method and long color stability allow for color comparison as the purification procedure is repeated; and it is possible that a quantitative colorimetric method may be developed for particular conditions, such as the control of manufacture or use where the same impurities are commonly present in about the same relative amounts.

This method serves a twofold purpose—purification of β-picoline and qualitative analysis of β-picoline for its common impurities.

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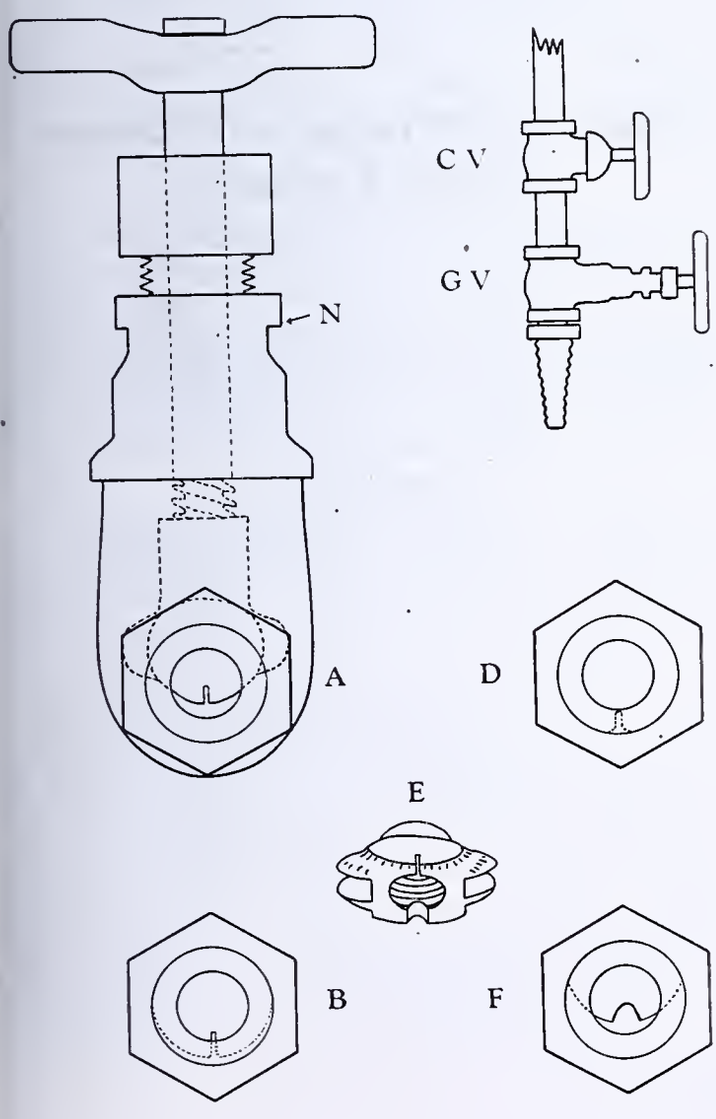
Modified Gate Valve for Control of Water Flow

G. ROSS ROBERTSON, University of California, Los Angeles, Calif.

LABORATORY workers have long deplored the fact that a water faucet, adjusted to low rate of flow, often shuts off the stream spontaneously. This treacherous habit unfortunately is a special failing of the "compression" valve, a device conceded to be the most satisfactory faucet yet developed. In the new arrangement shown in the figure, the regular compression valve, *CV*, with washer, is retained for leakproof on-and-off service. Adjustment to low rate of flow is delegated to a second device, the modified gate valve, *GV*.



In the standard commercial gate valve, *A*, a brass gate normally rises and falls across the water stream with rotation of the left-hand-threaded shaft and wheel handle. As shown in the separate view of the gate, *E*, a narrow slot is cut in the extreme edge of the gate. This slot permits the passage of a small but very definite and constant stream—for example, at adjustment *B*. The stream is precisely adjustable between values fully double that at *B* down to position *D*, where the stream is reduced practically to zero flow. At the same time it is possible to open the gate to full flow as needed. The modified gate valve thus has a much wider range of usefulness than a needle valve which might serve at position *GV* to provide a small stream.

Since it is unnecessary and undesirable to have a narrow slot on both sides of the gate, the lower side (as of gate in position *E*) is cleared for unrestricted flow with a wide slot, whose operating position is seen at *F*, which merely shows the opposite threaded end of valve *A*. In steady service of one kind from day to day—for example, to a reflux condenser—little or no adjustment of *GV* is required, and thus undue wear is obviated.

MECHANICAL SPECIFICATIONS. A standard plumbers' brass gate valve, usually $^3/_8$ or $^1/_4$ inch ("iron-pipe-size") is selected, choice being based on maximum width of the annular ground contact surface of the gate. Such maximum width permits maximum length of slot with limit shown in *D*. The slot is cut with a fine jewelers' saw; a blade 0.31 mm. in width was found to be satisfactory. The resulting slot is of suitable width for use with water pressure of 60 pounds per square inch, or approximately 4 kg. per sq. cm. It should be noted that the opposite and supposedly identical contact faces of a gate are often of varying annular width, or overlap. The wider, with the greater overlap, is selected for the narrow slot, *B*, and the narrower, also cut with the saw, for *F*.

Since this mechanical alteration requires disassembling of the gate valve, inexperienced workers are warned that nut *N* must not be opened while the valve is closed, lest the interior mechanism be sprung and the valve ruined. Instead, the gate is first screwed out about half way. The valve is now fastened securely in a vise, with vise jaws pressing upon the two threaded mouths of the device. Nut *N* may then be started without mechanical damage, and the parts separated by finger contact only.

BOOK REVIEWS

Official and Tentative Methods of Analysis. 6th edition. xii + 932 pages. Association of Official Agricultural Chemists, P. O. Box 540, Benjamin Franklin Station, Washington, D. C., 1945. Price, $6.25.

Ever since it was founded in 1884 the Association of Official Agricultural Chemists has devoted itself almost exclusively to studies in the field of methodology. Beginning with the standardization of methods for the analysis of commercial fertilizers, the scope of its work has expanded continuously to meet the ever-increasing needs of its members who are now concerned with the enforcement of laws and regulations relating to commercial fertilizers, stock feeds, food, drugs, cosmetics, insecticides, fungicides, caustic poisons, and other articles; or who are engaged in research in these and related fields.

Each succeeding edition of the association's text, which has come to be known by the short title "Methods of Analysis, A.O.A.C.", represents the elimination of obsolete or little-used methods, revisions and improvements of other methods, and the addition of many new procedures.

The 6th edition is notable for new chapters on cosmetics, gelatin, dessert preparations and mixes, enzymes, and extraneous materials in foods and drugs. A chapter on vitamins assembles under one head methods for vitamin assays previously scattered throughout the book under other titles. A mechanical improvement in the form of presentation is the use of Arabic numerals to designate chapters, with decimals for the subheadings therein. A special effort has been made to have the "selected references" cited at the end of each chapter show, as far as possible, the original work upon which the methods are based.

It is pertinent to note that these methods have acquired an added significance in recent years, since many of them are being incorporated in standards for food products promulgated under authority of the Federal Food, Drug and Cosmetic Act. This gives legal status to the procedures thus cited.

Laboratory workers in this country who are engaged in official work within the scope of the association's activities, and who comprise the active membership of the association, will welcome this new edition as an indispensable laboratory guide. So also will workers in like fields in foreign countries, among whom this text has come to have wide recognition and acceptance.

E. M. Bailey

Quantitative Organic Microanalysis. Edited by *Julius Grant*. 4th English ed. Based on the methods of Fritz Pregl. vii + 238 pages. The Blakiston Co., 1012 Walnut St., Philadelphia, Pa., 1946. Price, $5.00.

The third English edition of Pregl's classical book, published in 1937, was, like its two predecessors, simply a literal translation of the corresponding German edition. It was evidently felt that the Pregl text was sacrosanct.

Now comes Dr. Grant, with what is stated to be not just another and better translation, but rather something between that and a new and original treatise on organic microanalysis.

This seems to the reviewer to be a proper development. With all the great respect due to Pregl, there is no sense in continuing to pretend that he wrote the last word in organic microanalysis. The fact that he wrote the first words is sufficient to ensure him enduring fame. It is to be hoped that the next author to build upon the base established by Pregl will be even more emancipated than is Dr. Grant from an obligation to classicism.

Indeed, the reviewer's only real criticism of Dr. Grant's book is that he did not give nearly enough recognition to the hundreds who have followed Pregl. There was a time, not too many decades ago, when it would have been heresy to write a book on general analytical chemistry that was not described in the flyleaf as based on Fresenius. We have long since got over that; and yet Fresenius' fame as a pioneer has not dimmed. So let it be with Pregl!

The book under review has only 238 pages of text as compared with 271 in the previous edition. Despite this, more actual text is provided; this is achieved by better organization of material and by space economies in make-up. The older book had 27 chapters; the present, seven: Microchemical Balances, General Microanalytical Technique, Determinations of the Elements, Determinations of the General Groups, Determinations of the Physical Constants, Miscellaneous Physical Methods, and Calculations of the Results of Microanalyses.

The author has made a real attempt to discriminate among alternative methods, giving first place to those he believes to be the best, even though they originated outside the Pregl school. But, as stated earlier, the reviewer believes that this policy might have been pursued even more vigorously.

Specific mention should be made of Chapter III, wherein the sections of the determinations of the metals have been gratifyingly expanded. The book is modernized by brief mention, in Chapter VI, of the new techniques of electrometry, polarography, radioactive indicators, and ultraviolet fluorescence analysis. This is all to the good, as far as it goes; but it is hoped that the next edition will go much further. The indexes are much improved, and it is noted that the tables of gravimetric factors have been recalculated to take account of changes in atomic weights.

For the benefit of those not familiar with other editions of Pregl, we quote from Dr. Grant's Preface: "The needs of the beginner in microanalysis have been borne prominently in mind, although a background of knowledge of ordinary organic analysis is assumed."

This book is strongly recommended to all who have occasion to practice, teach, or study quantitative organic microanalysis.

B. L. Clarke

CORRESPONDENCE

Semimicrodetermination of Saponification Equivalent

Sir: Ketchum (*1*) has devised a semimicromethod for the determination of the saponification number (or the saponification equivalent). In comparing his method with ours (*2*) he states: "The procedure of Marcali and Rieman is limited to compounds which are readily soluble in alcohol and which can easily be saponified."

This statement is not correct, as is proved by the successful application of our method to eleven oils (*2*), of which only castor oil is readily soluble in alcohol.

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(2) Marcali, K., and Rieman, W., III. *Ibid.*, **18**, 144 (1946).

Kalman Marcali
William Rieman III
Rutgers University
New Brunswick, N. J.

• • •

Sir: In the sentence, "The procedure of Marcali and Rieman (*3*) is limited to compounds which are readily soluble in alcohol. . .", the word "readily" was employed only in the loose sense in which the word is often used. It was not a good choice of word, but the sentence does not alter the main theme that my method has a wider application than theirs, because of the solubility limitations imposed by their method. This is illustrated by the fact that the ketones which were successfully analyzed by my procedure could not be run by their method because of the solubility limitations. When these ketones were mixed with only twenty times their weight of alcoholic potash, they would not open the ring.

Donald Ketchum
Eastman Kodak Company
Rochester, N. Y.

INDUSTRIAL AND ENGINEERING CHEMISTRY
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WALTER J. MURPHY, EDITOR

Applications of Infrared Spectroscopy to DDT

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Infrared spectroscopy provides a valuable tool for the chemical characterization of technical DDT, including detection of the several isomers and impurities and quantitative estimation of DDT content. This technique has been applied in establishing the purity of commercial samples for use in formulation studies and in determining low concentrations of DDT in residues and dispersions.

UTILIZATION of dichlorodiphenyltrichloroethane (DDT) by the armed forces and increasing interest in potential postwar agricultural and domestic applications have focused attention on new methods for the identification and quantitative estimation of DDT. Various analytical methods have been devised for the quantitative determination of *p,p'*-DDT together with its isomers. Winter (*10*), Hall *et al.* (*5*), and Fahey (*3*) have employed methods of analysis depending upon the determination of halogens in organic compounds. Neal *et al.* (*7*) and Gunther (*4*) have described methods for determining DDT based upon dehydrohalogenation with alcoholic alkali and determination of the resulting HCl. Colorimetric methods have been reported by Schechter *et al.* (*8*, *9*) and Bailes (*1*). Cristol, Hayes, and Haller (*2*) have described a method for the determination of *p,p'*-DDT in technical DDT by recrystallization from saturated solutions of pure *p,p'*-DDT in aqueous ethanol.

Such methods are very useful but there is still a recognized need for a technique which will identify the several isomers of DDT and avoid complications arising when DDT is associated with other chlorine-containing compounds.

The term DDT is employed here in accordance with terminology in common usage in current literature publications: "The symbol 'DDT' is a contraction for dichloro-diphenyl-trichloroethane, the generic name of the active insecticidal principle. Theoretically there are forty-five possible dichlorodiphenyltrichloroethanes. However, the term 'DDT' is confined to the product obtained on condensation of chloral (or its alcoholate or hydrate) with chlorobenzene in the presence of sulfuric acid" (*6*). When referring to specific isomers of DDT, for example, *p,p'*-DDT is used to designate 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)-ethane and *o,p'*-DDT for 1,1,1-trichloro-2-*o*-chlorophenyl-2-*p*-chlorophenylethane.

In this laboratory, absorption spectroscopy has been successfully applied to the chemical characterization of DDT in connection with the development of special formulations for use by the armed forces. It has been shown that infrared spectroscopy provides a means of determining both the purity of DDT and the presence of relative proportions of the isomers and impurities which may occur along with *p,p'*-DDT.

It is well known that certain chemical structural units possess characteristic, isolated, infrared absorption frequencies. By study of reference samples of DDT and selected isomers, absorption bands may be assigned to the DDT molecule and to the isomers and by-products associated with commercial DDT. Critical examination of the absorption spectrum makes it possible to determine qualitatively the purity of a given sample of DDT and also the nature of the impurities. A quantitative analytical method for DDT purity and concentration may be devised on this basis. Both procedures have been used in the current research for characterizing commercial DDT's and for estimating DDT in spray compositions.

APPARATUS

The infrared spectrograph used in this work was built in 1940 in the physics shops of the University of Michigan under the supervision of H. M. Randall. It is a rock salt prism spectrometer of conventional optical design, using a 15-cm. rock salt prism and an off-axis parabolic mirror of 1-meter focal length. A record of per cent transmission versus wave length is recorded directly by means of a periscopic beam-splitting device and a recording potentiometer.

The spectrograph is calibrated in terms of wave lengths by running the spectra of standard substances such as ammonia (Figure 1), carbon dioxide, and water vapor. The instrumental quality permits a precision in measuring wave lengths of absorption bands of ±0.01 micron or better, although the practical accuracy in assigning wave lengths in routine work is usually not greater than ±0.1 micron. All wave lengths except those in Figure 1 are therefore expressed in the nearest 0.1 micron.

In studying DDT, carbon disulfide was used as the solvent. Rock salt cells, 5 or 20 mils thick, were used to hold the DDT solutions.

The volume of DDT solution prepared was usually about 5.0 ml., but less than 0.2 ml. is required to fill the absorption cell. The infrared absorption spectrum can be obtained from as little as 1 mg. of DDT if the volume of the solution is restricted to 0.2 ml.

About 45 minutes are required to record the complete spectrum over the range 1 to 15 microns. If only the range 7 to 12 microns is examined, a record can be made in about 10 minutes.

SOURCE OF REFERENCE COMPOUNDS

PURE *p,p'*-DDT (m.p. 108.6–109.5° C. corrected). U. S. Public Health Service; a sample purified by Merck & Co. for the Insect Control Committee of the Office of Scientific Research and Development.

o,p'-DDT (m.p. 73–74° C.). Grasselli Chemicals Department, E. I. du Pont de Nemours & Company, Cleveland, Ohio.

m,p'-DDT (liquid at room temperature). Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Beltsville, Md.; a sample furnished by M. S. Newman, Ohio State University.

1,1-DICHLORO-2,2-BIS(*p*-CHLOROPHENYL)ETHYLENE (m.p. 88–89° C. corrected). Prepared in this laboratory.

BIS(*p*-CHLOROPHENYL)SULFONE (m.p. 148–149° C. corrected). Prepared in this laboratory.

2,2,2-TRICHLORO-1-(*o*-CHLOROPHENYL)ETHYL-*p*-CHLOROBENZENESULFONATE (m.p. 105–106° C.). Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Beltsville, Md.; a sample from P. D. Bartlett, Harvard University.

1,1-DICHLORO-2,2-BIS(*p*-CHLOROPHENYL)ETHANE (*p,p'*-DDD) (m.p. 108–109.5° C.). Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Beltsville, Md.; a sample furnished by H. S. Mosher, Pennsylvania State College.

These reference compounds were studied without purifying to constancy of infrared spectrum.

Table I. Key Infrared Absorption Bands for DDT and Related Materials

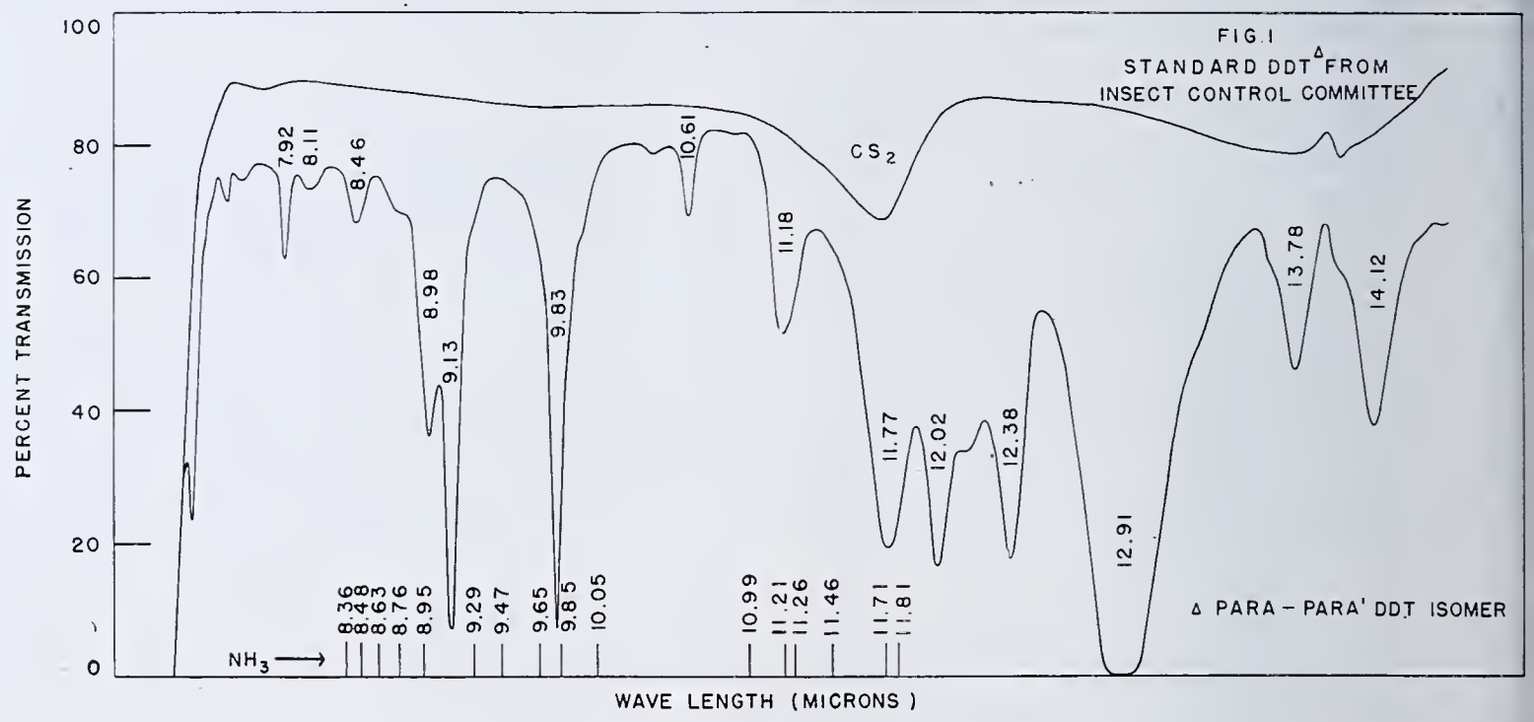
Figure	Compound	Wave Length, Microns
1	*p,p'*-DDT	9.1, 9.8, 12.9
2	*o,p'*-DDT	9.6, 13.3
3	*m,p'*-DDT	10.9
4	1,1-Dichloro-2,2-bis(*p*-chlorophenyl)ethylene	10.2
5	Bis(*p*-chlorophenyl)sulfone	8.6
6	2,2,2-Trichloro-1-(*o*-chlorophenyl)ethyl-*p*-chlorobenzenesulfonate	8.4, 10.1
11	*p*-Chlorophenyl group	9.0, 9.1
12	*o*-Chlorophenyl group	9.4, 9.6

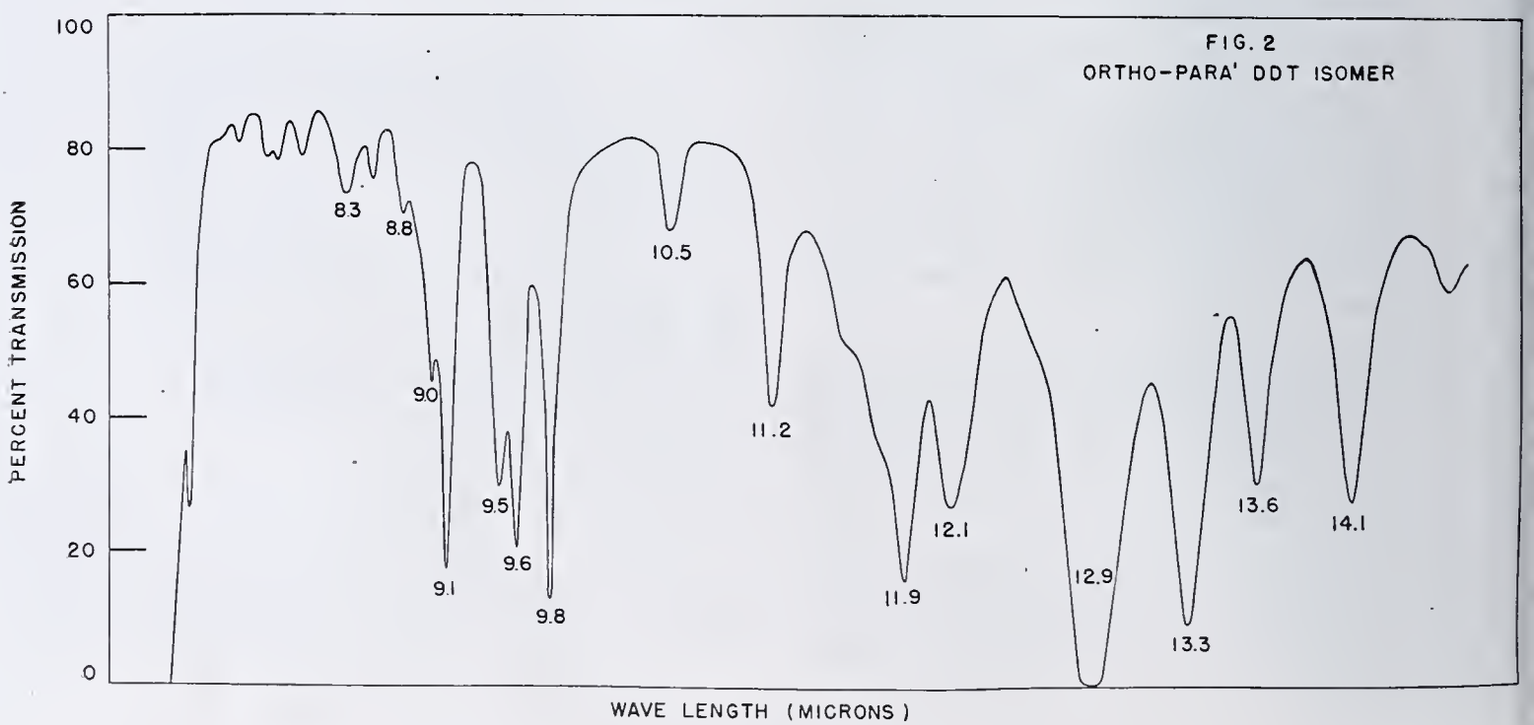
SPECTROSCOPIC STUDIES OF DDT AND RELATED COMPOUNDS

The characterization of absorption bands by assignment to structural units frequently occurring in commercial DDT was accomplished by the study of a group of reference compounds structurally related to DDT. The spectra of pure *p,p'*-DDT, the *o,p'*- and *m,p'*-isomers, and by-products encountered in commercial DDT are discussed in the following sections. A summary tabulation of key absorption bands for each compound is given in Table I.

PURE *p,p'*-DDT. A sample of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane, purified by recrystallization by Merck and Co. and selected by the Insect Control Committee as a comparison standard for purity, was examined spectrographically. The infrared absorption curve from 7 to 14.5 microns is shown by the tracing of the original record in Figure 1. The spectrum of the solvent, carbon disulfide, and the position of the ammonia absorption bands are also included for reference. Intense absorption bands were recorded at 9.1 and 9.8 microns which were later found to be bands common to the DDT isomers and impurities containing a *p*-chloro substituted phenyl grouping. The bands at 9.1 and 9.8 microns were assigned to the *p*-chlorophenyl groups by comparison with *p*-chlorotoluene (Figure 11). A third intense band at 12.9, when resolved at low concentrations, proved to be a doublet.

o,p'-ISOMER OF DDT. The absorption spectrum of 1,1,1-trichloro-2-*o*-chlorophenyl-2-*p*-chlorophenylethane is shown in Figure 2. In addition to strong absorption bands at 9.1 and 9.8 microns, there is a band of similar intensity at 9.6 which is not present in pure *p,p'*-DDT. This band is assigned to the *o*-chlorophenyl group by comparison with *o*-chlorotoluene (Figure





2). A strong band at 13.3 microns also characterizes this compound.

m,p'-Isomer of DDT. Strong absorption at 10.9 microns was the outstanding characteristic of 1,1,1-trichloro-2-*m*-chlorophenyl-2-*p*-chlorophenylethane (Figure 3). Major absorption bands for the *p*-chlorophenyl group occurred at 9.1 and 9.8 microns. Bands at 9.6 and 13.3 suggest the presence of some ortho isomer as an impurity in this sample.

Ethylene Analog of DDT. One of the more probable products of degradation of *p,p'*-DDT is the dehydrohalogenation compound, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene. The infrared absorption spectrum of this material (Figure 4) showed an intense band at 10.2 microns in addition to the bands at 9.1 and 9.8 microns.

Commercial By-products of DDT. An impurity found in commercial DDT which arises as a by-product in the manufacturing process is bis(*p*-chlorophenyl)sulfone. The infrared spectrum of this material (Figure 5) showed intense bands at 7.5 and 8.6 microns and an absorption band of lesser intensity at 7.8 microns. Minima at 9.1 and 9.8 were also present.

The absorption curve of 2,2,2-trichloro-1-(*o*-chlorophenyl)-ethyl-*p*-chlorobenzenesulfonate, another by-product in DDT manufacture, showed minima at 9.1, 9.6, and 9.8 microns (Figure 6). Other absorption bands are at 8.4 and 10.1 microns.

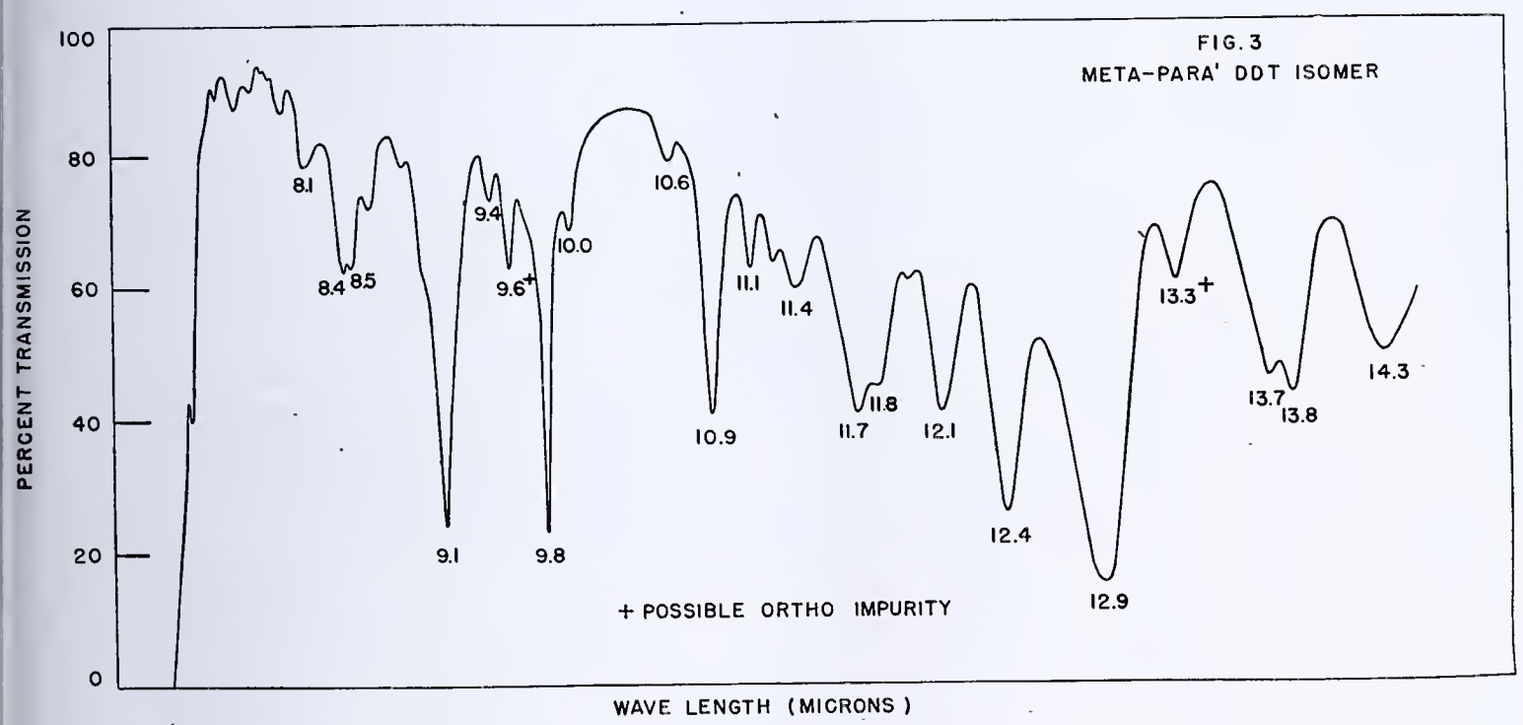
The spectrum of 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (*p,p'*-DDD) was very similar to that of pure *p,p'*-DDT except for displacements of some of the bands (Figure 7).

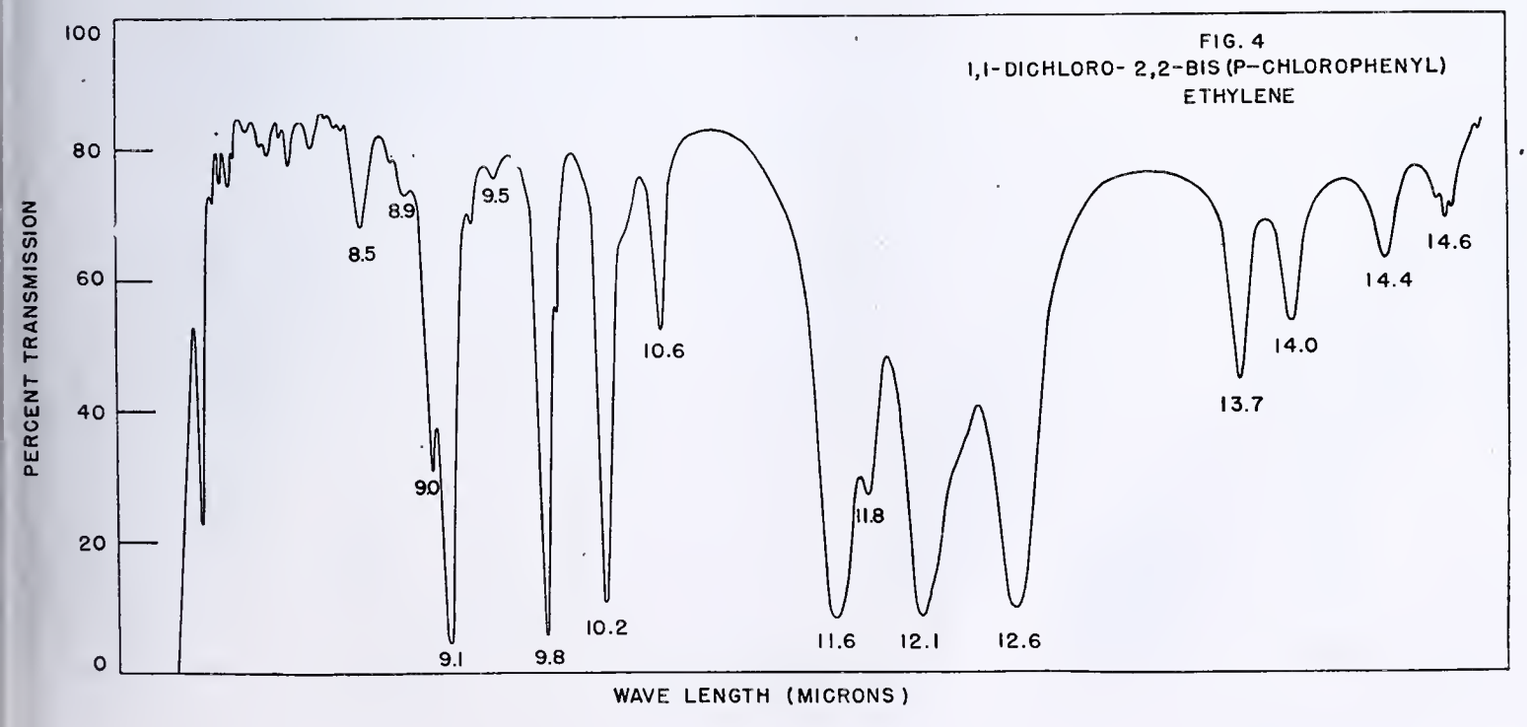
BASIS FOR ASSIGNMENTS

The designation of certain strong absorption bands as key absorption bands for the compounds involved was made on the basis of a direct comparison of the spectra of the compounds. The assignment of the band at 9.1 microns to the *p*-chlorophenyl group and the band at 9.6 microns to the *o*-chlorophenyl group is based solely on the spectra of *p*-chlorotoluene and *o*-chlorotoluene. Although these assignments seem reasonable and consistent, they are tentative and should be restricted to the DDT system and not applied to para and ortho configurations generally without further evidence.

SENSITIVITY

The limiting sensitivity of the method to *p*- and *o*-chlorophenyl groups is about 0.5 mole per cent under the conditions used—namely, 1-gram sample in 5 cc. of carbon bisulfide in a 5-mil cell. If higher sensitivity for the determination of *o,p'*- or *p,p'*-isomers





is desired, it can readily be attained by using a thicker cell or by using the bands at 12.9 and 13.3 microns.

APPLICATIONS OF INFRARED TECHNIQUE

The practical value of the infrared absorption method for the study of DDT is illustrated by several applications.

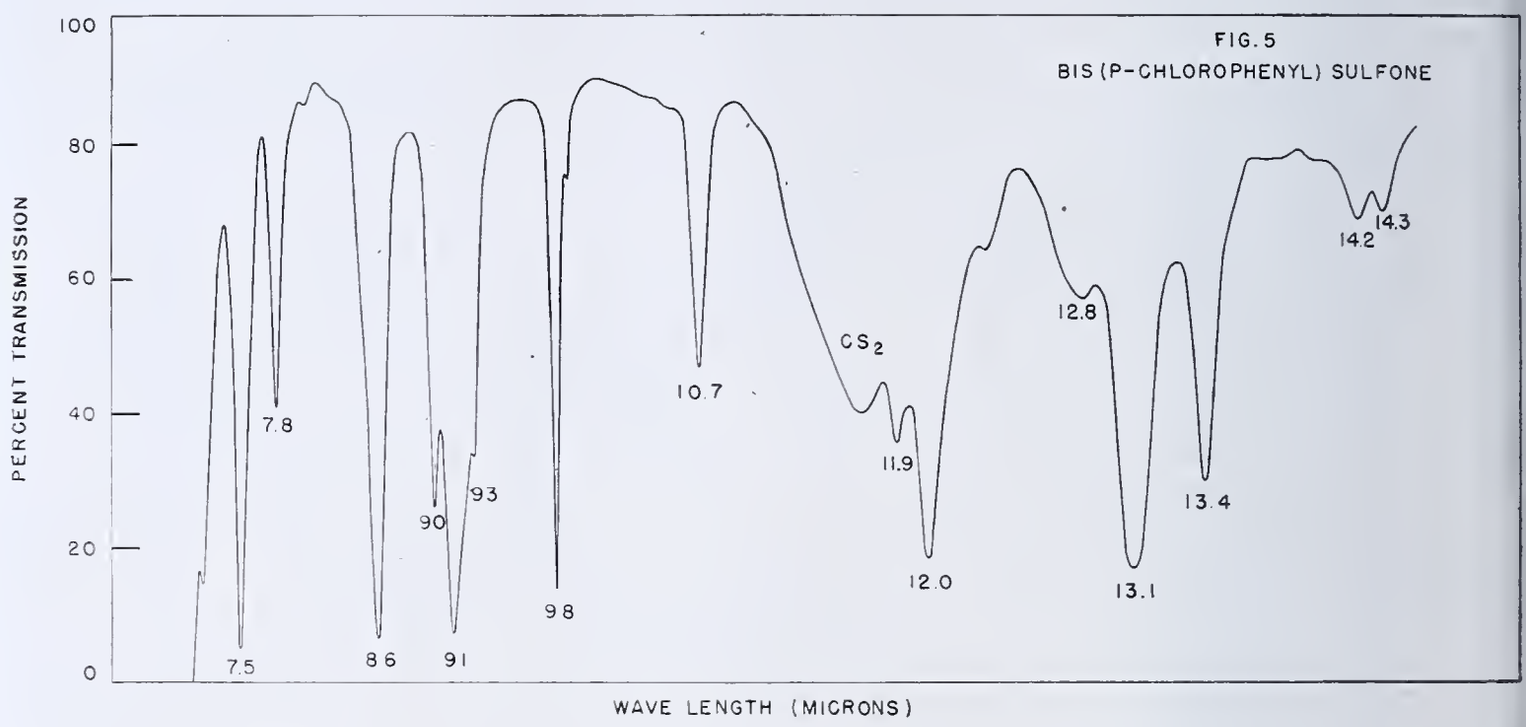
The purity of laboratory recrystallized DDT has been confirmed by the use of the infrared absorption technique. Comparison of the spectra of this material and the standard DDT showed by the absence of bands at 9.6 and 13.3 microns that the recrystallized material was free of *o*-isomer (Figure 8).

Two commercial DDT samples of a special grade from the same manufacturer gave melting points of 106–107° and 102.5–104.5° C. The higher melting sample, when subjected to a high-temperature test for caking tendency, stood up under these conditions while the lower melting DDT in the same system failed completely. The question arose as to whether the caking tendency of the latter was due to residual solvent, the presence of which was indicated by strong odor, or to other impurities in the DDT. Spectrographic examination requiring less than 30 minutes showed the presence of the *o,p'*-isomer. The presence of the *o,p'*-isomer in the low melting sample (Figure 10) is shown by definite absorption at 9.6 and 13.3 microns, while the higher melting sample (Figure 9) shows only trace absorption at these wave lengths.

The application of the infrared absorption method of determining qualitatively the purity of DDT was further demonstrated by examination of a group of commercially available DDT samples discussed in the following section. The transmission curves of these samples, on the basis of the absorption bands assigned to the isomers and by-products, indicate the relative prominence of the known impurities.

COMMERCIAL DDT SAMPLES

As an illustration of the applicability of the method in assaying the purity of commercial samples, absorption coefficients taken from the recorded spectra of a number of commercial samples are tabulated in Table II. The isomers and impurities



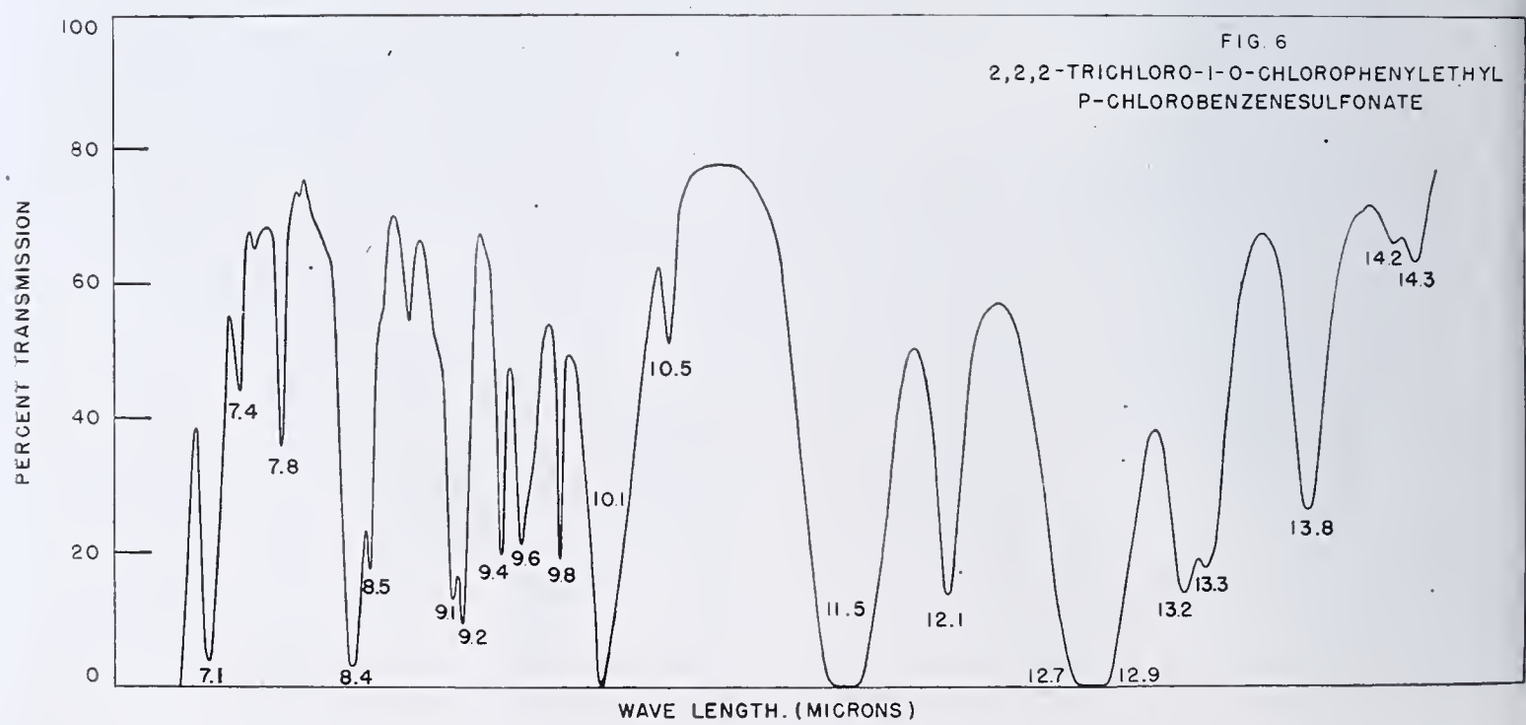


Table II. Absorption Coefficients for Impurities in Commercial Samples of DDT

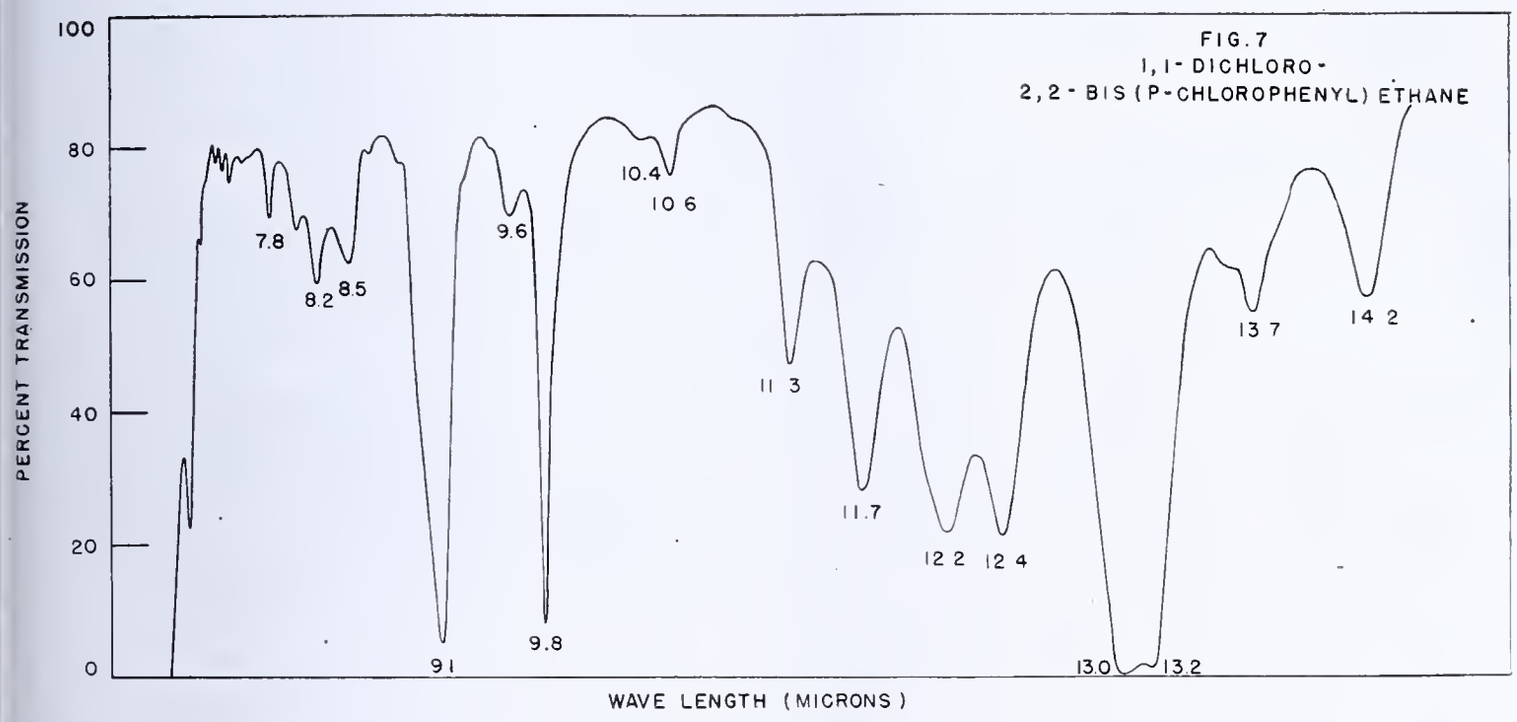
Sample[a]	o,p'-DDT 9.6	o,p'-DDT 13.3	2,2,2-Trichloro-1-(o-chlorophenyl)ethyl-p-chlorobenzenesulfonate, 8.4	Bis(p-chlorophenyl)-sulfone, 8.6
A	0.15	..	0.05	0.06
B	0.17	..	0.11	0.01
C	0.15	..	0.09	0.01
D	0.01	..	0.00	0.00
I	0.14	..	0.08	0.02
II	0.14	..	0.09	0.02
IIIA	..	0.04	0.03	0.00
IIIB	0.03	0.20	0.04	0.00
IV	0.15	..	0.09	0.00
V	0.15	..	0.07	0.01
VI	0.06	..	0.06	0.12
VII	0.12	..	0.06	0.03
VIII	0.15	..	0.16	0.02
IX	0.11	..	0.09	0.00
X	0.14	..	0.07	0.02
XI	0.13	..	0.18	0.03

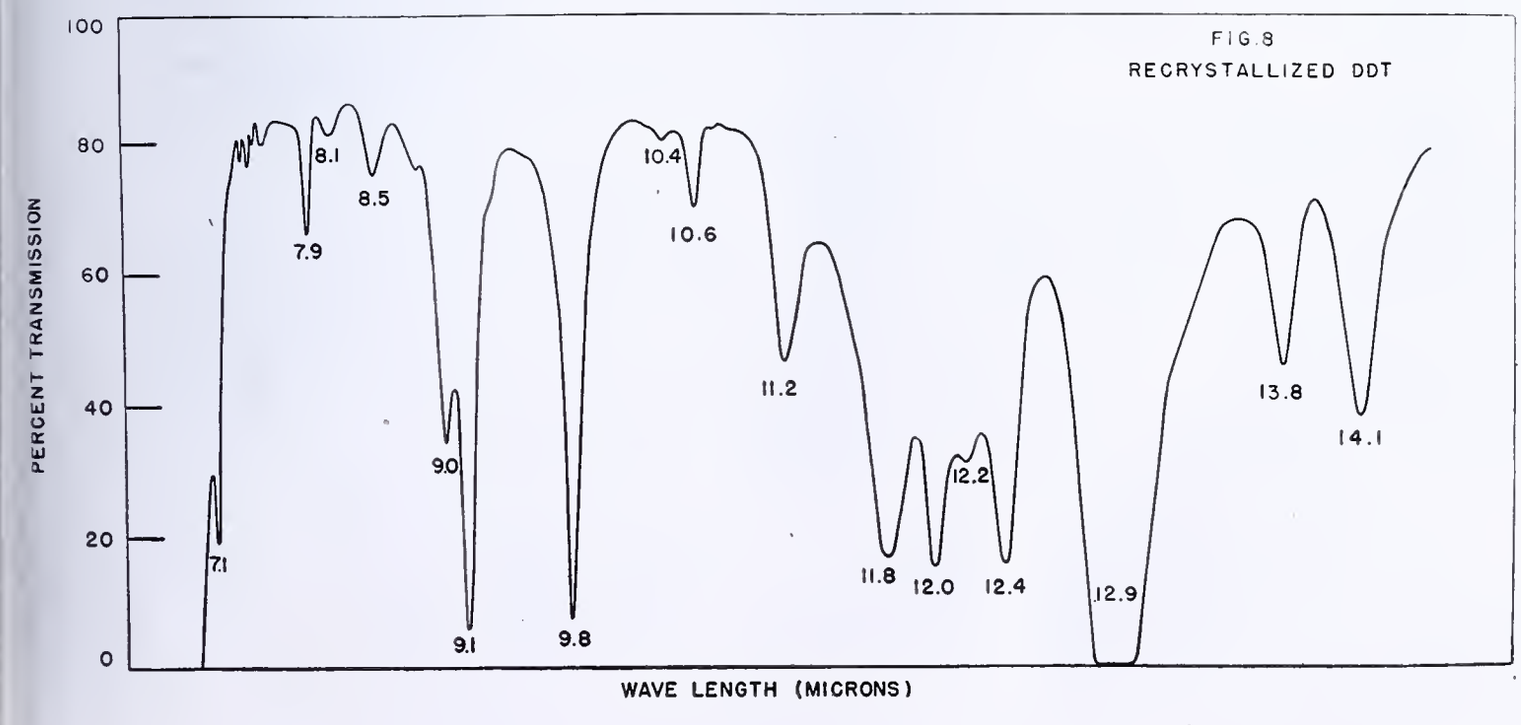
[a] From various manufacturers. D, IIIA, and IIIB are different lots of a special grade from one producer; A and VII, B and V, and C and XI represent pairs from three different producers.

most commonly encountered in technical DDT, in addition to p,p'-DDT, are (1) the o,p-isomer, (2) bis(p-chlorophenyl)sulfone, and (3) 2,2,2-trichloro-1-(o-chlorophenyl)ethyl-p-chlorobenzenesulfonate, with a trace of the m,p'-isomer in some samples.

The absorption coefficient, expressed in Table II as $\log_{10} I_0/I$ where I_0 = transmitted radiation at zero concentration and I = transmitted radiation of the sample at the given wave length, is a linear measure of the concentration of the impurities present and may be calibrated in terms of mole per cent by studying known mixtures.

An examination of these data shows that there are obvious differences, apparent from a qualitative study of the original records, in the relative concentrations of the various impurities in the several samples. For example, samples D and IIIA appear to have the least amounts of the three impurities listed above. Sample XI has a high content of 2,2,2-trichloro-1-(o-chlorophenyl)ethyl-p-chlorobenzenesulfonate, while sample VI is high in bis(p-chlorophenyl)sulfone content. For a finer distinction between samples low in o-isomer, such as IIIA and IIIB, the band at 13.3 microns was used.





QUANTITATIVE DETERMINATION OF DDT

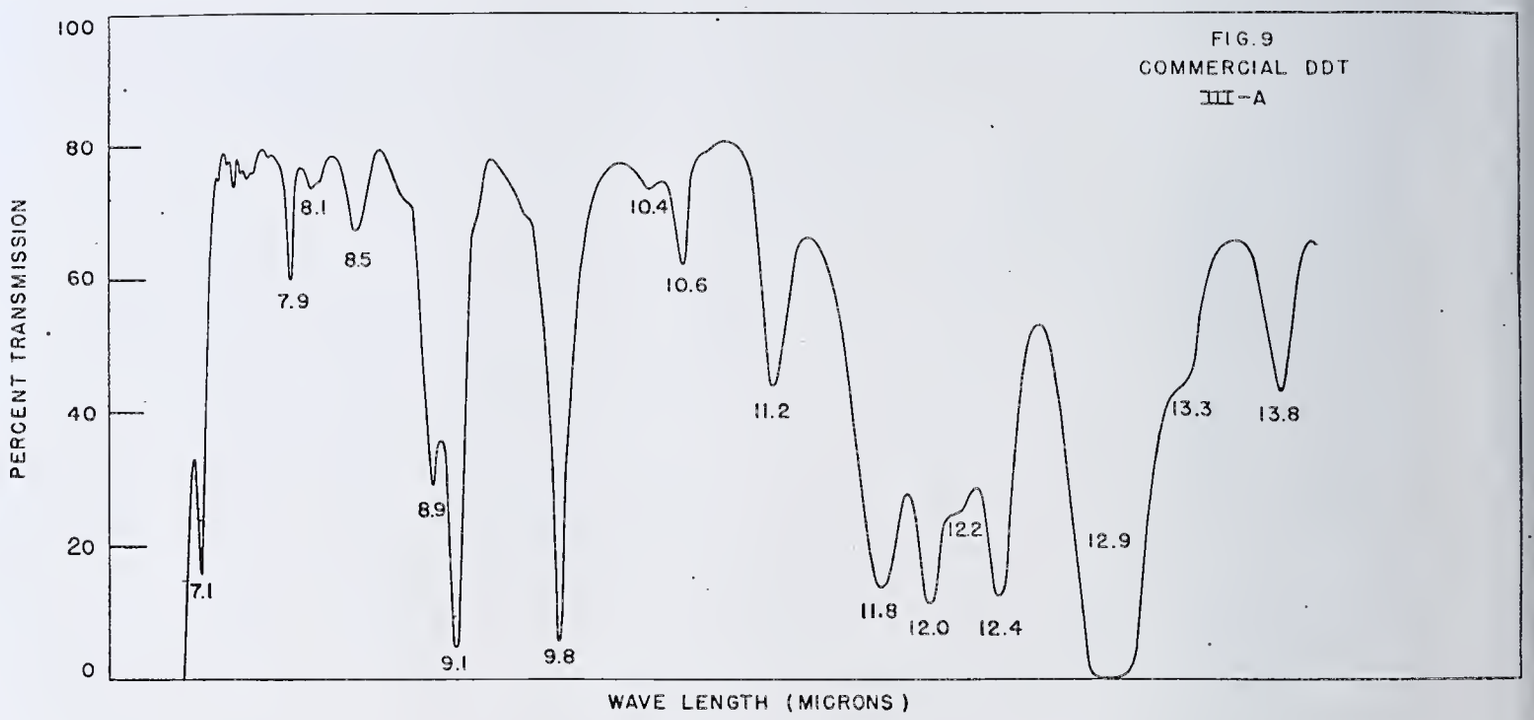
Infrared absorption provides the basis for an analytical procedure for determining concentration of DDT, which has the following advantages: (a) rapidity of analysis, results available in less than 30 minutes; (b) analysis of samples too small for gravimetric or volumetric methods; (c) preservation of sample.

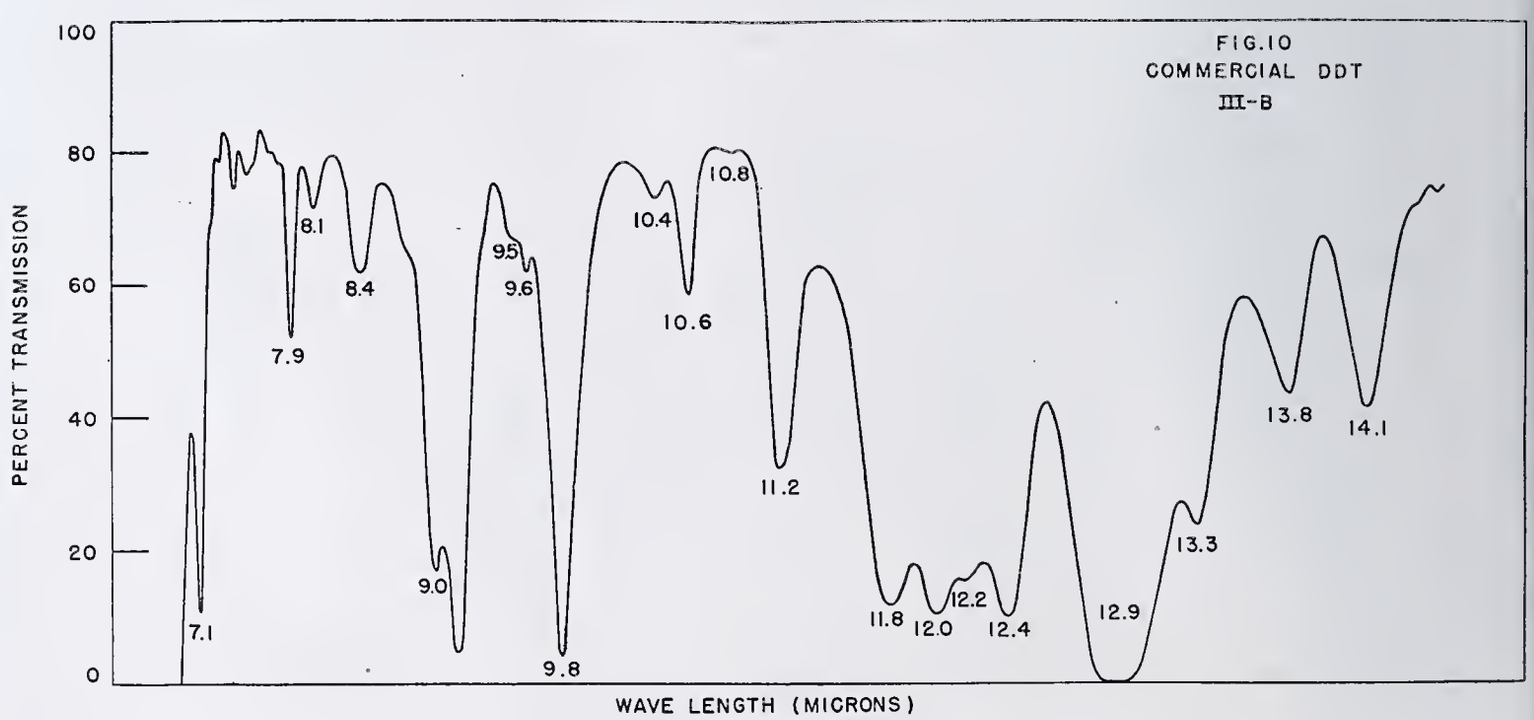
The method has been used experimentally in this research in analyzing dilute aqueous suspensions of DDT used in tests against insects. The suspensions were prepared in such a manner that calculation of composition was not possible; the infrared method was therefore utilized. A quantity of suspension sufficient to yield 10 to 100 mg. of DDT was evaporated to dryness. The residue was dissolved in sufficient carbon bisulfide to give a solution containing from 0.2 to 1.2% DDT. The concentration of DDT in the carbon bisulfide was determined accurately by infrared absorption, and from this figure the concentration of DDT in the original water suspension was calculated. It is necessary, of course, in such cases to prepare a standard curve of transmission versus concentration by which transmission of the unknown sample can be related to its concentration.

The same procedure can be used to analyze spray residues recovered from leaf surfaces, fruits, etc. Thus, in this investigation, concentration of residual spray on walls has been determined by washing DDT from the surface and analyzing for DDT spectroscopically. In such cases it is desirable to run a "blank" consisting of the solvent extract of unsprayed surfaces like those being examined to ensure that interfering substances are not being extracted from these surfaces.

In carrying out these analyses, a DDT of predetermined composition was used and its treatment and exposure were such that the composition was not altered. The only object was to learn the amount of such DDT present in a given sample. For such work, the 9.8-micron band was used and only the transmission at this single band was required for analysis of the samples.

A more complicated situation will be met in some cases. DDT of unknown history or material subjected to conditions possibly causing chemical change may be submitted. The quantitative determination of such materials will require more time and results will be less accurate than in the simple case cited above. It is necessary first to obtain the infrared absorption spectrum of the sample in order to determine whether impurities—e.g., isomers, by-products, or degradation products—are present. It has been shown previously that these extraneous materials have characteristic absorption bands. By means of standard transmission versus concentration curves prepared for the extraneous materials, their concentration in a particular sample can be determined. Likewise the total concentration of material showing absorption at 9.8 microns can be measured. The concentration of p,p'-DDT in the sample can be determined by dif-





ference. The same procedure is useful for determining the proportions of the several isomers in DDT. By use of standard curves prepared from pure samples of the isomers, the concentration of *m,p'*-DDT or *o,p'*-DDT in the sample can be measured. The concentration of the *p,p'*-isomer will then be calculated by difference, using the 9.8-micron band for determining total DDT in the sample.

STATUS

Although methods based on infrared absorption have proved valuable for research purposes in this investigation, their development has not been carried to the point of setting up detailed procedures which can be applied directly in control. It is believed that the basis for development of such procedures has been laid by the work described above.

ACKNOWLEDGMENTS

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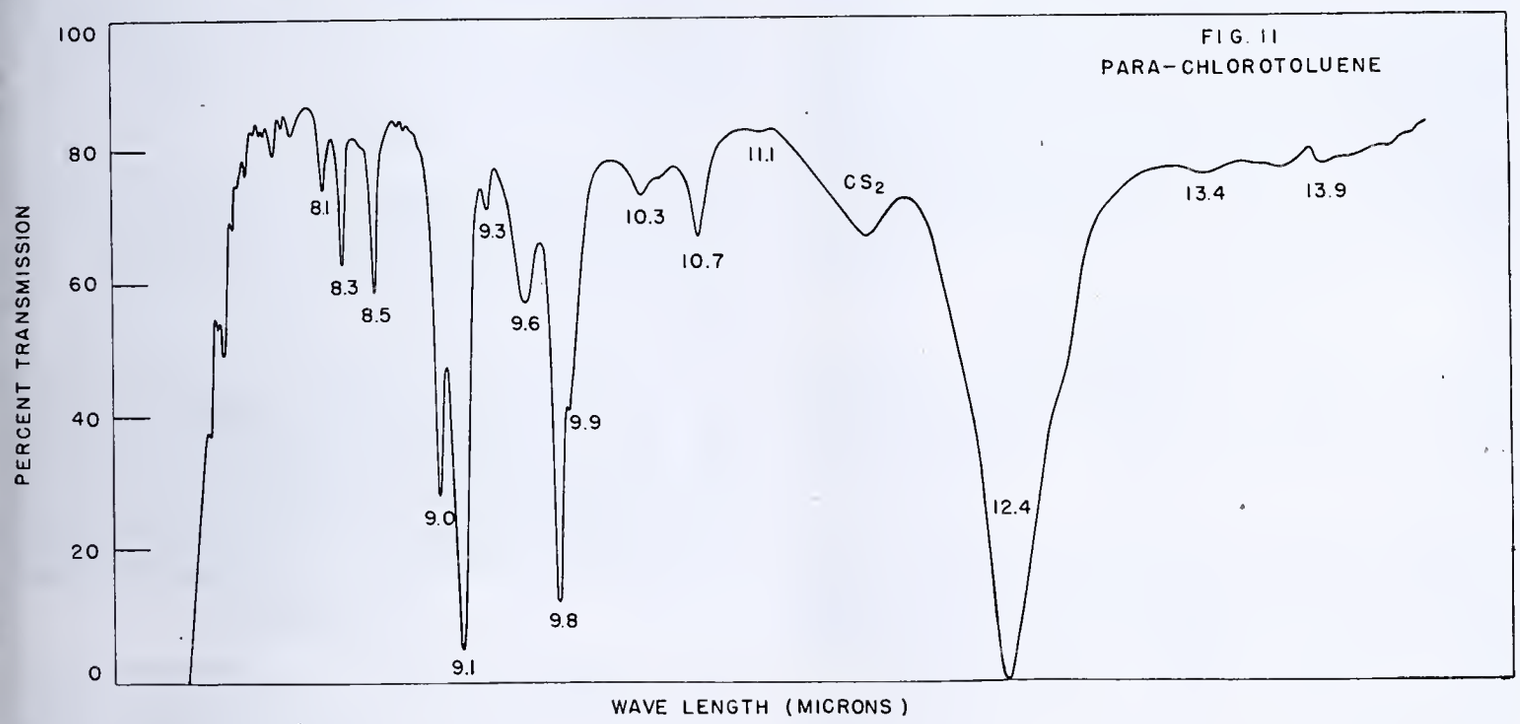

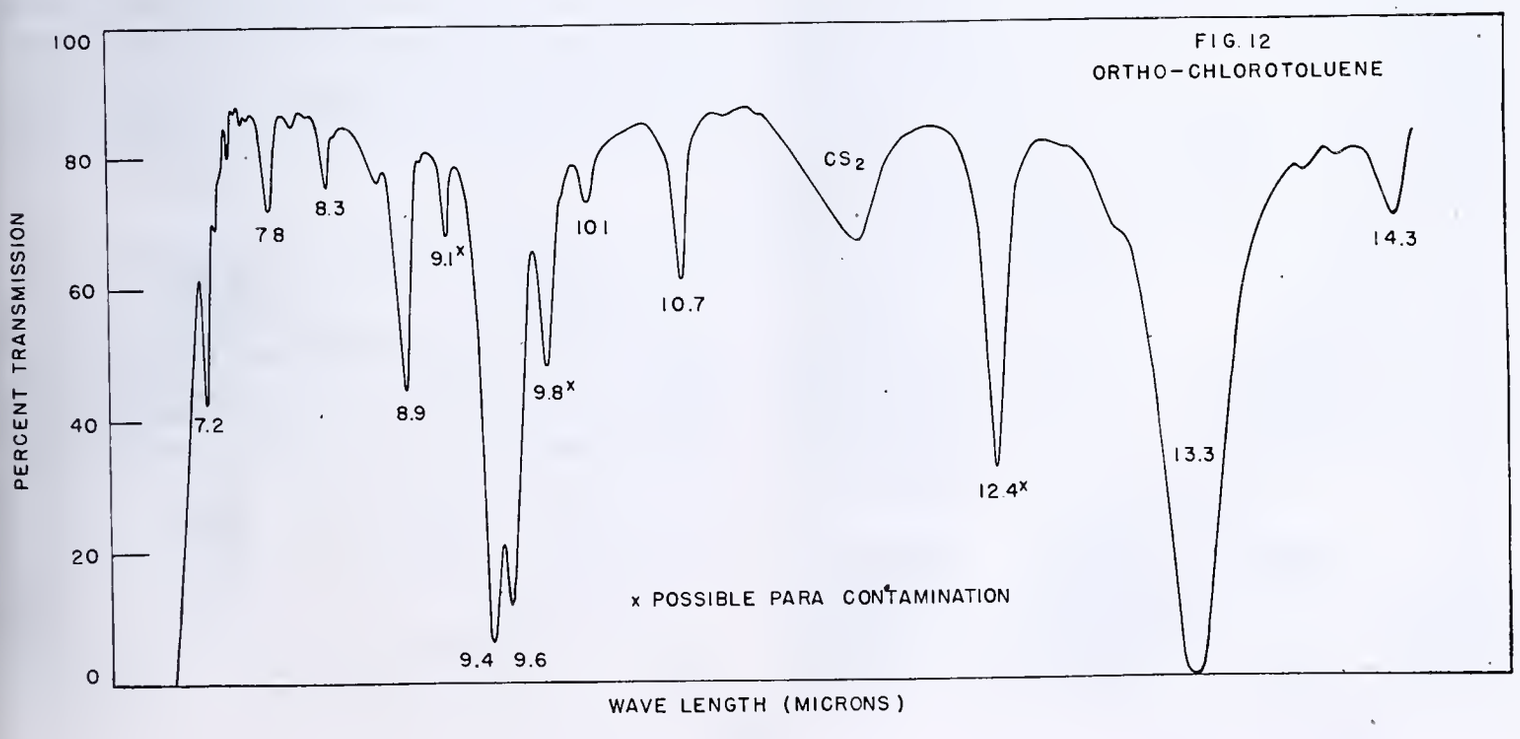

Routine Analysis of Sodium-Potassium Alloys

S. L. WALTERS AND R. R. MILLER

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A volumetric method for the determination of potassium in sodium-potassium alloys is described. A sample of the alloy is obtained in an ampoule in such a way that contact with moisture and oxygen is prevented. The sample thus obtained is reacted with absolute alcohol under neohexane and subsequently titrated with standard acid. Alloys of higher than 92% potassium content are solid at room temperature and are analyzed by means of their freezing points. An equation is given for the freezing point curve. Accuracy of ±0.1% potassium is readily obtainable. Liquid alloys can be analyzed in less than 1 hour and solid alloys in about 5 minutes.

IN A problem involving the production of metallic potassium, an urgent need arose for a method of determining potassium when alloyed with sodium, which could be used for routine control analyses. The usual gravimetric methods involving the formation of the chloroplatinate, cobaltinitrite, or perchlorate were investigated and found sufficiently accurate, but too slow. These methods were used only when impurities were such that the volumetric method described below was inapplicable.

The method reported here was designed for alloys of sodium and potassium with little or no impurities. In the presence of foreign elements results are vitiated by multiplied errors in calculations. The alloys analyzed in this laboratory contained over 99.9% sodium and potassium.

APPARATUS AND PROCEDURE

The most difficult part of an alloy analysis of this type is obtaining a clean, oxide-free sample of known weight. The apparatus shown in Figure 1 was developed to enable a weighed sample of clean alloy to be taken, avoiding contact with oxygen or moisture.

A sample (approximately 30 grams) of the alloy was drawn into the sample bulb by evacuating through the stopcock and drawing the alloy from the original container into the sample bulb through a glass tube fitted with a ground joint. The sample bulb was then fitted to the sampling device, which was clamped in such a position that it could be rotated to raise either end. A weighed thin glass ampoule was placed in the sampler; the system was evacuated through stopcock *A* and filled with nitrogen through stopcock *B*. The nitrogen was purified by passing over copper turnings maintained at 425° C. and a drying tube filled with Anhydrone. The sample bulb and sampler were warmed with a flame to permit free flow of the alloy. The apparatus was then tipped so that the alloy could flow from the sample bulb into the capillary. A slight vacuum was applied to draw the desired weight of sample (approximately 1 gram) into the alloy ampoule. Nitrogen was admitted to force the excess alloy out of the capillary back into the sample bulb. The sampler was returned to the horizontal position and the alloy ampoule was removed, quickly sealed in a gas flame, then cooled, and weighed. In this manner an accurate weight of clean, oxide-free alloy was obtained.

The weighed ampoule of alloy was placed in a 32 × 300 mm. test tube and covered with neohexane. The neohexane used was purified by washing first with sulfuric acid, then with alkali, and distilling over sodium. The ampoule was broken with a glass rod and absolute alcohol added dropwise to the hexane, decomposing the sample. The alcohol was added slowly to avoid loss of sample by entrainment in the hydrogen evolved. The decomposition tube was kept in a water bath to minimize overheating.

When the sample had been completely decomposed it was washed out with distilled water into a 250-ml. Erlenmeyer flask and titrated with 1 *N* hydrochloric acid, using phenolphthalein as indicator. At the end point the hexane was evaporated on a hot plate and the titration continued to the methyl orange end point. The use of phenolphthalein reduces danger of overstepping the methyl orange end point and speeds the titration.

CALCULATIONS

Calculations were made in the following manner:

Let

x = weight of potassium in sample in grams
w = weight of sample in grams
ME = milliequivalents of hydrochloric acid

$$ME/1000 = x/39.1 + (w - x)/23$$

Solving the equation for x gave

$$x = 2.43\,w - 0.0559\,ME$$

An operator with a little practice can analyze an alloy in less than an hour with an accuracy of ±0.1% potassium. Considerable care must be exercised in following the procedure to obtain this precision. The most frequent errors are those caused by impure hexane, oxide film, and loss of sample in transferring. Because of the hazardous nature of the materials involved, safety goggles should be worn at all times during sampling and decomposition of the alloy.

SOLID ALLOYS

Alloys containing more than 92% potassium are solid at room temperature; therefore a rapid and convenient method of determining their composition is by their freezing points. Since the published data are not well defined in this region (*2*, *3*), this part of the freezing point curve has been determined at this laboratory.

Freezing points were taken directly in the sample bulb (Figure 1) by inserting a calibrated thermometer graduated in 0.2° C. The alloy was warmed until liquid, and the bulb placed in a beaker of magnesia and allowed to cool slowly. The first thermal arrest was noted, the thermometer removed, and the sample analyzed as described above. When per cent potassium was plotted against temperature of the freezing point a straight line was obtained in the region from 92 to 100% potassium (by weight). The equation of this line can be represented by the relationship

$$y = 0.259\,t + 83.5$$

where y is weight per cent potassium and t is the corrected temperature in degrees Centigrade.

The freezing point method of analysis is not readily extendable to alloys below 92% potassium because of the decreased sharpness of the first thermal arrest.

The freezing point of pure potassium was found to be 63.7° C. This value is 1.2° higher than the usually reported value, but is

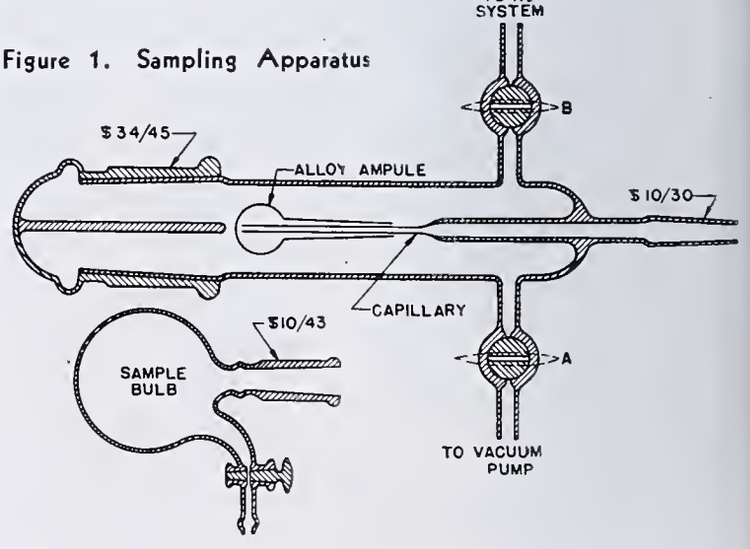

Figure 1. Sampling Apparatus

in good agreement with the value of 63.65° reported by Edmondson and Egerton (*1*). A considerable amount of potassium has been made in this laboratory which analyzed 99.9+% potassium and gave a corrected freezing point of 63.7° C.

SUMMARY

A rapid method for the routine analysis of alloys of sodium and potassium is presented. Alloys of greater than 92% potassium can be analyzed in less than 5 minutes while those under 92% can be analyzed in less than an hour. With the exercise of reasonable care results should be accurate to within ±0.1% potassium. The freezing point of molten potassium has been determined as 63.7° C.

ACKNOWLEDGMENT

The authors gratefully acknowledge the services of C. A. Kraus of Brown University, from whose original work this method was developed, and A. Spencer Lehmann and C. R. Witschonke of Brown University and later this laboratory, who contributed by both suggestions and much of the work on the production of the alloys.

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Determination of Ethylene Chlorohydrin

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A CONVENIENT method for determining ethylene chlorohydrin in binary mixtures is the refractometric procedure as proposed by Berry (*1*) and by Gomberg (*2*). This method, however, is not specific for ethylene chlorohydrin and is, therefore, unsuitable in certain cases. Other methods mentioned in the literature, such as the colorimetric procedure of Sapadinsky (*4*) or one mentioned in Berry's (*1*) paper without reference or details and involving reflux of the sample with silver nitrate in nitric acid solution, were found too unreliable. Miller and Denny (*3*) used a method in which the ethylene chlorohydrin is hydrolyzed by barium hydroxide on standing overnight, and the chlorine in the resulting barium chloride is determined as an indication of the ethylene chlorohydrin concentration.

It is a well-established fact that ethylene chlorohydrin can be easily hydrolyzed by refluxing with caustic, and it was thought that this reaction might be made the basis of a more specific and reliable method. While it was not possible to make the caustic consumed a direct measure of the ethylene chlorohydrin, determination of the chloride ion of the alkali chloride which is formed proved to be successful.

APPARATUS AND REAGENTS

Apparatus required includes an electric (or other) hot plate provided with a rack for support of reflux condensers, reflux condensers with standard-taper ground-glass joints, 300-ml. Erlenmeyer flasks with standard-taper ground glass joints to fit the above condensers, a 50-ml. buret, and assortments of volumetric flasks and pipets.

The reagents are 5% aqueous potassium hydroxide, C.P. nitric acid, 0.1 *N* ammonium thiocyanate, 0.1 *N* silver nitrate, and ferric ammonium sulfate indicator (3.5 grams of ferric ammonium sulfate, 10 ml. of water, and 2 ml. of 6 *N* nitric acid).

PROCEDURE

From 3 to 5 grams of sample are weighed accurately into a 100-ml. volumetric flask and diluted with water to 100 ml. From this solution aliquots are measured into 300-ml. Erlenmeyer flasks. The volume of the aliquots should be such that they do not contain more than 350 mg. of ethylene chlorohydrin. The aliquots are refluxed with 50 ml. of 5% potassium hydroxide solution for one hour. The contents of the Erlenmeyer flasks are then cooled to room temperature, and 10 ml. of concentrated nitric acid, followed by 50 ml. of 0.1 *N* silver nitrate solution, are added. After addition of 2 ml. of ferric ammonium sulfate indicator, the unreacted silver nitrate is titrated with ammonium thiocyanate. The reacted silver nitrate is a measure of the ethylene chlorohydrin in the aliquot. One milliliter of 0.1 *N* silver nitrate is equivalent to 8.05 mg. of ethylene chlorohydrin. A blank is run on 50 ml. of 5% potassium hydroxide solution, omitting the refluxing.

If the sample should be dissolved in a nonaqueous, water-insoluble solvent, the sample weight should be such that not more than 350 mg. of chlorohydrin are actually present and the entire sample should be refluxed. This amount of ethylene chlorohydrin (350 mg.) is the safe maximum amount which can be determined with 50 ml. of 0.1 *N* silver nitrate.

Solvents and compounds which contain loosely combined halogens interfere with the determination of chlorohydrin. In case any of these compounds are present, it is suggested that the chlorohydrin be extracted with two equal volumes of water and be determined in each extract. If X_1 and X_2 represent the grams of ethylene chlorohydrin found in the respective extracts, the grams of ethylene chlorohydrin in the original sample, X, can be calculated from

$$X = \frac{X_1^2}{X_1 - X_2}$$

Some chlorinated solvents have an appreciable solubility in water. Since these are usually highly chlorinated, even a relatively low solubility may introduce considerable errors. Chloroform belongs in this class. In such instances distillation will probably be the best means for separating ethylene chlorohydrin from the interfering substances.

If ethylene chlorohydrin is to be determined in presence of inorganic chlorides, distillation will also be the most convenient means for separation. Care must be taken that sufficient water be present or be added, so that the constant-boiling mixture (42.5% ethylene chlorohydrin, 57.5% water; boiling point 96° C. at 760 mm.) will carry over the ethylene chlorohydrin with the first portions of the distillate.

The accuracy and reproducibility of the method described are evidenced by Table I, which shows a few of the many results obtained on aqueous and nonaqueous solutions of ethylene chlorohydrin.

Table I. Ethylene Chlorohydrin

Found, Mg.	Present, Mg.	Solvent
26.4	27.0	Water
59.5	59.4	Water
117.3	118.3	Water
177.7	178.0	Water
235.5	237.0	Water
357.8	357.6	Water
196.3	195.9	Xylene
322.5	324.0	Xylene
355.9	359.0	Carbon tetrachloride plus xylene 1 to 1

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Colorimetric Determination of Local Anesthetic Compounds

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A NUMBER of esters of *p*-aminobenzoic acid are employed in therapeutics as local anesthetic agents. Since these compounds contain a diazotizable aryl amine group, the authors have adapted the Bratton and Marshall method (*2*) of determining sulfanilamide derivatives to the colorimetric determination of these anesthetic agents. In this method, the primary aryl amine is diazotized and coupled with *N*-(1-naphthyl) ethylenediamine to yield a colored azo dye. Use of this coupling component results in a simpler and more sensitive method than when β-naphthol is employed as described by Willstadt (*7*) and Eissner (*3*). Although the method is not specific when two or more primary aryl amines are present as a mixture, such mixtures are seldom encountered in practice, and the diazotization procedure is generally more specific and simpler than the bromination methods used for assay of local anesthetic agents (*1*, *5*, *6*).

The official U.S.P. XII drugs, benzocaine, procaine hydrochloride, butacaine sulfate, and butyl aminobenzoate were chosen for investigation, although other local anesthetic agents, such as larocaine, tutocaine, monocaine, and panthesine may be determined in a similar manner. Cocaine, metycaine, alypine, stovaine, diothane, nupercaine, and phenacaine do not yield azo dyes and hence do not interfere in the determination. Likewise, epinephrine and ephedrine, which are often used in conjunction with local anesthetic agents, do not interfere.

REAGENTS

Standard solutions of anesthetic compounds.

0.001% solutions of benzocaine, procaine hydrochloride, butyn sulfate, and butesin in distilled water were prepared with the aid of a minimal quantity of dilute hydrochloric acid if necessary.

10% ammonium hydroxide solution.

4 *N* sulfuric acid.

Chloroform, reagent grade.

All other reagents necessary were prepared according to the specifications of Bratton and Marshall (*2*) with the exception of ammonium sulfamate which was replaced with undenatured 95% per cent ethanol as recommended by Lee, Hannay, and Hand (*4*).

DEVELOPMENT OF COLOR

Pipet 1, 2, 3, 4, and 5 cc. of the respective standard solutions of the anesthetic compounds into 50-cc. volumetric flasks each containing 5 cc. of 4 *N* sulfuric acid. Add 1 cc. of 0.1% sodium nitrite solution to each flask and allow to stand 3 minutes, then add 5 cc. of 95% ethanol and allow to stand for 2 minutes. Add 1 cc. of 0.1% *N*-(1-naphthyl) ethylenediamine solution, shaking after each addition, and make up to volume with distilled water. The color is developed rapidly and may be read in a photoelectric colorimeter after several minutes.

PHOTOMETRIC MEASUREMENTS

Absorption curves with a single peak absorption at 545 mμ, using a Coleman Model 10-S spectrophotometer, were noted.

Per cent transmittances of the solutions were determined with an electrophotometer (Fisher Electrophotometer, Fisher Scientific Co., Pittsburgh, Pa.), using a filter transmitting at 525 mμ and plotted against concentration on semilog paper. A plot of the per cent transmission thus obtained resulted in a smooth curve which was essentially a straight line in conformity with Beer's law.

AQUEOUS AND INJECTABLE SOLUTIONS

Dilute the solution with distilled water so that the final dilution contains approximately 10 micrograms of *p*-aminobenzoic acid ester per cc. Use a 5-cc. aliquot of this dilution for the development of color. Read the per cent transmittance on a photoelectric colorimeter and determine the concentration from the standard curve.

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OINTMENTS

Weigh accurately a sample of ointment calculated to contain approximately 0.1 gram of *p*-aminobenzoic acid ester. Dissolve in 50 cc. of petroleum ether and extract with five 10-cc. portions of 1% sulfuric acid. Filter the combined acid extracts through a moistened filter paper into a 100-cc. volumetric flask and add sufficient distilled water through the filter to bring the volume to 100 cc. Transfer 5 cc. of this solution to a 500-cc. volumetric flask and make up to volume with distilled water. Use a 5-cc. aliquot of this dilution for the development of color. Read the per cent transmittance on a photoelectric colorimeter and determine the concentrations from the standard curve.

COMPRESSED TABLETS

Reduce 10 or 20 tablets, whichever is most convenient, to a fine powder by grinding in a mortar. Accurately weigh a portion of this powder calculated to contain approximately 0.1 gram of the *p*-aminobenzoic acid ester. Suspend or dissolve the powder in 50 cc. of distilled water in a separatory funnel, make slightly alkaline with 10% ammonium hydroxide, and extract with five 10-cc. portions of chloroform. Pass the combined chloroform extractions through a funnel containing a small pledget of cotton previously moistened with chloroform and wash the funnel and cotton with an additional 10 cc. of chloroform. Evaporate the chloroform on a water bath with the aid of a gentle air blast impinged on the surface of the solution. When the chloroform solution has reached a volume of 5 cc., add 5 cc. of ethyl alcohol and evaporate to dryness at a temperature not exceeding 50° C. To the dry residue add 5 cc. of alcohol and then 50 cc. of 1% sulfuric acid and warm on a water bath to 50° C. Transfer to a 100-cc. volumetric flask with the aid of distilled water and make up to volume with distilled water. Transfer 5 cc. of this solution to a 500-cc. volumetric flask and make up to volume with distilled water. Use a 5-cc. aliquot of this dilution for the development of color. Read the per cent transmittance on a photoelectric colorimeter and determine the concentration from the standard curve.

BLOOD AND SPINAL FLUID

To 4 cc. of trichloroacetic acid in a 15-cc. centrifuge tube, add 1 cc. of blood, serum, plasma, or spinal fluid dropwise, shaking after the addition of each drop. Centrifuge for 3 minutes and transfer 1 cc. of the clear, supernatant liquid to a 100-cc. volumetric flask. Make up to volume with distilled water and use a 5-cc. aliquot of this dilution for the development of color. Read the per cent transmittance on a photoelectric colorimeter and determine the concentration from the standard curve. If higher concentrations are expected, other suitable dilutions may be used.

This method cannot be used if sulfonamides are present, since they will interfere by giving the same color reaction.

DISCUSSION

The color produced in this reaction develops rapidly and reaches its full intensity in from 1 to 2 minutes. It is stable for at least 4 hours, with the exception of butyn sulfate, in which case it begins to fade in about one hour. Exposure of the colored solutions to strong light should be avoided, since this causes fading.

The best working range for this method was found to be from 10 to 50 micrograms for the electrophotometer used.

A number of determinations carried out on known amounts of the compounds investigated indicate that a maximum error of about 2% may be expected in the recommended working range.

The efficiency of the method for pharmaceutical preparations depends upon the thoroughness of extraction and the complexity of the mixture. As pointed out above, diazotizable aryl amines interfere, and the possibility of their presence in complex mixtures must be considered.

SUMMARY

A rapid colorimetric method for the determination of local anesthetics of the *p*-aminobenzoic acid ester type consists of

diazotizing the amino group and coupling with *N*-(1-naphthyl)-ethylenediamine dihydrochloride. Data resulting from determinations of procaine hydrochloride, benzocaine, butyn sulfate, and butesin indicate a maximum error within 2%.

Procedures for the determination of these compounds in pure systems, in biological fluid, and in various pharmaceutical preparations are given. Aromatic amino compounds, notably sulfonamides, interfere.

ACKNOWLEDGMENT

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Molecular Weight and Mercaptan Content of Mixtures of Primary Mercaptans

Gravimetric Determination

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The mercaptan sulfur content of a mixture of primary mercaptans is determined by titration with silver nitrate, and the weight of silver mercaptide formed during the titration is determined. From these data the average molecular weight of the mercaptans and the percentage of mercaptans are calculated. The percentage of nonmercaptan material is found by difference.

COMMERCIAL mixtures of primary mercaptans may contain varying amounts of alkyl disulfides, alkyl halides, alcohols, etc., as impurities. A method was desired for determining the average molecular weight of the mercaptan fraction of the mixture, rather than the average molecular weight of the whole mixture as would be determined by cryoscopic measurements. The percentage of nonmercaptan material is often of interest in following the fractionation of mixtures.

Knowing the weight of silver mercaptide formed from a known number of moles of mercaptan (determined by silver nitrate titration), the molecular weight of the mercaptan fraction can be calculated.

PROCEDURE

Dissolve an accurately weighed sample of 0.3 to 0.5 gram of mercaptan in 400 ml. of absolute ethanol in a 600-ml. beaker. Add 6 ml. of concentrated ammonium hydroxide, and titrate with 0.1 *N* aqueous silver nitrate to an amperometric end point (*1*), adding the reagent dropwise with efficient stirring. If more than 0.1 ml. of reagent in excess is added, it is advisable to back-titrate with a very dilute solution of mercaptan in alcohol.

After allowing the precipitate to settle, pour the clear supernatant liquid through a weighed fritted-glass crucible. Do not transfer any precipitate. Pour the suspension of precipitate into centrifuge tubes and centrifuge at high speed for 3 minutes. Wash the precipitate quantitatively into the centrifuge tubes with absolute ethanol, loosening the precipitate with the aid of a rubber policeman. Stir up the precipitate in the centrifuge tubes and centrifuge again. Repeat the operation of adding ethanol, stirring, and centrifuging until the precipitate has been washed three times. Transfer the precipitate into the crucible using a minimum quantity of ethanol and allow to drain.

Dry the precipitate for 2 hours at 60° to 70° in a vacuum oven and weigh.

CALCULATIONS

$$M = \frac{1000\ W}{A \times N} - 106.9$$

$$\text{Per cent RSH} = \frac{A \times N \times M}{S} \times \frac{1}{10}$$

Per cent nonmercaptan materials = 100 − per cent RSH

where M = average molecular weight of mercaptans in mixture
W = weight of silver mercaptide
A = ml. of silver nitrate used
N = normality of silver nitrate
S = weight of sample

DISCUSSION

Because of the difficulty of washing the precipitated silver mercaptides, the precipitation is carried out with an exactly equivalent amount of silver nitrate. The amperometric method of Kolthoff and Harris (*1*), in which aqueous silver nitrate is added to an ammoniacal alcoholic solution of mercaptan, was found convenient. The potentiometric method of Tamele and Ryland (*2*), using alcoholic silver nitrate, could also be used. An accidental excess of silver can easily be removed by adding a very dilute alcoholic solution of mercaptan of known concentration.

In order to prevent occlusion of mercaptan with the silver mercaptide it was found necessary to use sufficient alcohol and to titrate with silver nitrate of such concentration that the final composition of the solvents was 95% alcohol. To prevent local excess of water, the silver nitrate should be added slowly with efficient stirring. The use of alcoholic silver nitrate (*2*) might be advantageous.

Considerable difficulty was encountered in filtering the silver mercaptide. The mercaptide settled readily, leaving a clear supernatant liquid, but the precipitate clogged the pores of the filter. The best filtering medium found was a fine fritted-glass crucible under efficient suction. Since washing the precipitate on the filter was too slow to be practical, centrifuging and decantation were used.

The precipitate was found to decompose when dried at 100° C., but at temperatures up to 70° C. no change in weight upon prolonged heating was observed, even though the color darkened to a deep orange. Mercaptides containing an excess of silver nitrate were less stable than carefully washed samples.

The procedure given above is intended for mixtures of primary mercaptans. When applied to tertiary mercaptan mixtures, two difficulties were encountered. The silver mercaptides of mixed tertiary mercaptans were found to be soluble in alcoholic solution. By adding enough water after the addition of silver nitrate to make the final solution 75 to 85% alcohol, the mercaptides were precipitated. However, the mercaptides were liquid at room temperature, making the filtering and washing operations very difficult. By carrying out the titration at 0° to −15° C., the

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Table I. Molecular Weight of Primary Mercaptans

Mercaptan	Mercaptan Sulfur Calcd. %	Mercaptan Sulfur Found %	Molecular Weight Calcd.	Molecular Weight Found	RSH %
$C_8H_{17}SH$	21.91	21.00	146.3	146.5	96.0
$C_{10}H_{21}SH$	18.39	18.15	174.3	175.2	99.2
$C_{12}H_{25}SH$	15.84	15.74	202.4	202.3	99.3
$C_{14}H_{29}SH$	13.91	13.80	230.4	232.8	100.2
$C_{16}H_{33}SH$	12.40	12.35	258.5	260.5	100.4

mercaptides were precipitated as solids which could be filtered and washed (with 70% alcohol) without centrifuging. Unfortunately, even the low-temperature technique did not permit filtration without centrifuging when applied to primary mercaptans. The accuracy of the modified method for tertiary mercaptans was not established, although the precision was comparable to that of the primary mercaptan method.

ACCURACY AND PRECISION

A series of straight-chain primary mercaptans containing eight to sixteen carbon atoms per molecule was analyzed. The mercaptans were prepared from carefully fractionated alkyl bromides which were obtained from alcohols derived from fats. The samples were believed to be of the correct molecular weight, but it was suspected that they contained small amounts of nonmercaptan impurities.

The results are given in Table I. Each result is the average of at least three determinations.

From the results in Table I it is concluded that the method yields the correct molecular weight to an accuracy of 1 to 2 units of molecular weight (0.5 to 1%).

Duplicate determinations in general were reproducible to ±1 unit of molecular weight.

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Determination of Acid in the Presence of Aluminum

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Of the methods proposed for estimation of free acid in aluminum salts, probably the most satisfactory are those in which the acid is titrated with standard alkali after conversion of the aluminum into a stable complex ion. From the Craig procedure, which involves the fluoride complex, an accurate method has been developed for the determination of acid in solutions in which the concentration of acid is high with respect to that of aluminum. A volumetric method for the analysis of hydrous alumina, embodying the improved procedure, is described.

LITERATURE and data relative to the determination of acid in the presence of aluminum salts have been in existence for nearly a hundred years. [References to much of the early literature on the subject are given by Beilstein and Grosset (*1*), Ivanov (*10*), and Kolthoff and Furman (*12*).] Because of the industrial importance of aluminum sulfate, most of the work has been devoted to the study of methods of determining free acid in samples of this salt.

The direct titration of the free acid in aluminum sulfate by means of standard base has been widely advocated. Indicators that have been recommended as suitable for use in such a titration in the presence of the salt include Congo red (*21*), methyl orange (alone, *2*, and with Martius yellow, *19*), thymol blue (*3*), sodium alizarin sulfonate (*17*), and the coloring matter of peonies (*17*). Procedures employing differential back-titrations for the determination of both aluminum and free acid in aluminum sulfate have also been suggested (*2*, *6*). In some of the methods the use of titration reference standards containing pure aluminum sulfate at the appropriate concentration is recommended; such a procedure should be regarded as essential because the pH of a solution of a pure aluminum salt is dependent on the concentration (and on the temperature and on the nature of the anion). The outstanding disadvantage of methods involving a titration in the presence of the aluminum salt is that, because of hydrolysis of the aluminum ion, the indicator does not undergo a sharp color change.

Other procedures, in which the aluminum is precipitated before the titration of the free acid, have been proposed. In particular, methods based on the insolubility of ammonium alum in ethyl alcohol (*1*, *11*) and on the precipitation of aluminum by potassium ferrocyanide (*10*, *25*) have been recommended. In some of these procedures the acid is titrated in the presence of the precipitated aluminum; in others the precipitate is first removed by filtration. The precipitation methods are troublesome and rather inaccurate.

Certain other methods that have been studied (*13*, *14*, *15*, *23*) are, like the foregoing ones, subject to criticism on one ground or another.

The most satisfactory methods for this determination, and the ones that have wide applicability to aluminum salts in general, achieve removal of the interference of aluminum by combining it in the form of a complex ion. The free acid may then be determined by titration with standard alkali; the iodometric method has also been proposed (*8*). Complexes that have been suggested as sufficiently stable to prevent significant hydrolysis of the aluminum ion are those formed with oxalate (*8*, *9*) and fluoride ions [a method has been investigated (*24*) that depends on the prevention, by the use of the citrate complex, of one third of the hydrolysis normally arising from aluminum on titration with hydroxide]. The technique involving the formation of a fluoride complex is recommended by, among others, Scott (*18*), Rosin (*16*), and Thorpe (*22*).

The fluoride method for the determination of free acid was probably first proposed by Craig (*5*). The aluminum salt is dissolved in water, potassium (*5*, *18*), or sodium fluoride (*16*, *22*) solution (previously adjusted to be neutral to phenolphthalein), is added in sufficient excess to convert the aluminum into the hexafluoaluminate complex, and the free acid is then titrated by means of standard alkali to a phenolphthalein end point. Bromothymol blue has also been used (*7*) as an indicator in connection with the fluoride method. A method for the simultaneous determination of free acid and aluminum ion, making use of the fluoride complex, has been proposed recently (*20*).

MODIFICATION OF FLUORIDE METHOD

In the course of a study of the reactivity of hydrous alumina toward acids, it was necessary to determine the decrease in the normality of a number of acid solutions that had been in contact with hydrous alumina for various periods of time. The aluminum concentration of the solutions to be analyzed varied from 0 to a maximum value of approximately 0.03 *M*, whereas the acid

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Table I. Accuracy of Conventional[a] Fluoride Method

Concn. of Al^{+++}, Moles/Liter $\times 10^4$	Concn. of F^-, Moles/Liter $\times 10^2$	Molar ratio, F^-/Al^{+++}	Titer of HCl (± 0.0001 N)	Recovery of Acid ($\pm 0.1\%$)
42	12.5	30	0.0939	98.0
83	25	30	0.0936	97.7
100	30	30	0.0921	96.1

[a] "Conventional" means a method in which the fluoride is added before the start of the titration. Details of such a method—e.g., concentration of base employed for the titration—vary from one worker to another (*5*, *16*, *18*)

Table II. Accuracy of Improved Fluoride Method

Concn. of Al^{+++}, Moles/Liter $\times 10^4$	Molar Ratio, F^-/Al^{+++}	Extent of Neutralization When KF Added ($\pm 0.1\%$)	Titer of HCl (± 0.0001 N)	Recovery of Acid ($\pm 0.1\%$)
111	30	97.0	0.0947	98.9
111	30	98.5	0.0953	99.5
111	30	99.5	0.0959	100.1
67	60	99.5	0.0959	100.1
67	30	99.5	0.0958	100.0
67	15	99.5	0.0959	100.1

concentration, in general, ranged from initial values of 0.2 or 0.1 N to normalities little more than half of these figures. Thus, although qualitatively the problem was similar to that of determining free acid in aluminum salts, the concentration ratios of acid to aluminum encountered in this work were relatively high.

Experiments using sulfuric, hydrochloric, nitric, or oxalic acid, together with dissolved aluminum chloride, showed that the conventional fluoride method (in which the fluoride is added before the alkalimetric titration) is not quantitatively accurate when the concentration of acid is high with respect to that of aluminum. For example, with an acid concentration of 0.07 N, an aluminum concentration of 5.6×10^{-3} M, and a potassium fluoride concentration of 1.7×10^{-1} M, the acid recovery was found with these acids to vary from 97.6 to 98.6%. The data in Table I further illustrate the degree of inaccuracy encountered. (The mole ratio of acid to aluminum in these experiments was several hundred times that encountered in aluminum salts where the mole ratio of free acid to aluminum may be of the order of 0.01.)

The solutions used in these titrations were prepared by adding to 20.00 ml. of standard hydrochloric acid solution sufficient aluminum chloride hexahydrate (recrystallized from an analyzed grade salt) and 1 M potassium fluoride solution (previously adjusted so as to give a very faint pink shade in the presence of phenolphthalein—i.e., neutral to phenolphthalein—and then stored in a wax-lined bottle) to give the concentrations noted in the table. The solutions were titrated in a paraffined beaker by means of standard 0.1 N sodium hydroxide solution to a phenolphthalein end point. The indicator blanks on water, potassium fluoride, and aluminum chloride plus potassium fluoride were identical. The concentration of hydrochloric acid in the 20.00-ml. sample (determined in both the absence and presence of potassium fluoride) was 0.0958 ± 0.0001 N.

Although the molar ratio F^-/Al^{+++} was constant in these experiments, the percentage of acid recovered decreased with increasing concentration of aluminum. This effect is very possibly due to increased occlusion of acid by the larger amount of alkali fluoaluminate precipitate at the higher aluminum concentrations. That the percentage recovery of acid is also a function of the molar ratio F^-/Al^{+++} was shown in a series of similar experiments; with this ratio varying from 7 to 30, and with the concentration of aluminum 0.01 M, the recovery varied from 99.1 to 96.1%.

If the low values obtained for acid in the presence of aluminum are due to losses by occlusion, this effect should be greatly lessened by adding the potassium fluoride, not at the beginning of the titration, but at a stage corresponding to almost complete neutralization of the acid. This modification of the method leads to accurate determinations of free acid in the presence of aluminum, as shown by the data in Table II.

At least with the concentrations used in the above experiments, the efficiency of the acid recovery is independent of the molar ratio F^-/Al^{+++} when the potassium fluoride is added at a stage of the titration corresponding to 99.5% neutralization. (A minimum molar ratio of 6 is, of course, necessary to convert the aluminum ion to AlF_6^{---}.)

Other experiments—for example, with oxalic acid and aluminum chloride—have confirmed the above finding that quantitative determinations of the acid may be carried out in the presence of aluminum, if addition of the potassium fluoride solution is delayed until neutralization is within a few tenths of 1% of completion.

In practice, one or two trial determinations will be necessary in order to ascertain the point at which the neutral potassium fluoride solution should be added. The writer has found it convenient to add the potassium fluoride at the start of the first trial titration (titrations are carried out in paraffin-lined beakers), and from the value obtained for the acid titer, tentatively assuming it to represent 98% of the true titer, to calculate the volume of alkali corresponding to 99.5% neutralization. The potassium fluoride is added after the addition of this volume of alkali in the titration of the second aliquot, and if the end point is not obtained within 0.5% of this value, the potassium fluoride point of addition is further adjusted before titrating remaining aliquot samples.

VOLUMETRIC ANALYSIS OF HYDROUS ALUMINA

When hydrous alumina is prepared by precipitation from an aluminum salt, it is normally contaminated with the anion of the salt used. No such anion, however, is present in aluminas prepared by the interaction of amalgamated aluminum metal with distilled water. If a weighed quantity of such a pure hydrous alumina is dissolved in an excess of standard acid, and the excess determined using the fluoride method to eliminate the interference of the aluminum, then the equivalents of aluminum present are given by the equivalents of acid neutralized. [For volumetric methods for aluminum, making use of the fluoride complex, see Snyder (*20*) and the references given by him, and the paper by Chernov and Nekrasov (*4*).] From the aluminum content, the composition of the alumina in terms of $Al_2O_3.xH_2O$ is readily determined. The calculation is facilitated by the use of the readily derived relationship:

$$F.W. = \frac{6000\,w}{(x - y)N}$$

where $F.W.$ = formula weight of $Al_2O_3.xH_2O$
w = weight of hydrous alumina sample
N = normality of standard alkali used
x = ml. of standard alkali required to titrate volume of acid used for dissolution of sample
y = ml. of standard alkali required to titrate excess acid after complete dissolution

The following procedure was used:

A 0.23-gram sample of powdered pure hydrous alumina was weighed to ± 0.1 mg. and transferred to a 125-ml. Erlenmeyer flask, 50.00 ml. of standardized 0.4 N sulfuric acid were added, and the mixture was boiled gently until solution was complete. During the dissolution period, which required 2 to 3 hours, the volume of the acid solution was maintained essentially constant (by additions of water), until toward the end when it was allowed

Table III. Sodium Hydroxide Required to Titrate Sulfuric Acid after Various Treatments

Direct Titration	Titration after Boiling		Titration after Addition of	
	2 hours	3 hours	15 ml. of neutral 2 M KF	$AlCl_3$ equivalent to 0.15 gram of Al_2O_3 and 15 ml. of 2 M KF
32.66	32.65	32.63	32.65	32.68
32.64	32.65	32.63	32.67	32.67
32.66	32.63	...	...	...

to decrease to approximately 25 ml. The solution was cooled to room temperature and the excess sulfuric acid present was titrated by means of standard alkali according to the improved fluoride method, using 15 ml. of 2 *M* potassium fluoride solution (neutral to phenolphthalein).

Table IV. Analyses of Hydrous Aluminas by Volumetric and Gravimetric Methods

	Sample 1		Sample 2	
	Gravimetric	Volumetric	Gravimetric	Volumetric
Formula weights	156.7	157.3	142.7	143.7
	157.1	157.5	142.2	143.7
	156.7	157.4	142.2	143.7
	156.9	...	142.3	143.9
Average formula weight	156.9	157.4	142.4	143.8
Average x in $Al_2O_3 \cdot xH_2O$	3.1	3.1	2.2	2.3

Tests showed that quantitative acid recovery was obtained for conditions similar to those of an analysis. In these control experiments, a constant volume of 0.3930 *N* sulfuric acid, diluted to 35 ml., was titrated by means of approximately 0.3 *N* sodium hydroxide after the treatments noted in Table III (the figures in this table refer to the number of milliliters of sodium hydroxide solution required).

A comparison of the results obtained using the volumetric method outlined above with those given by the gravimetric procedure (ignition to constant weight in platinum, using a Fisher blast burner) is given in Table IV. Both alumina samples had been prepared, one (sample 1) at room temperature and the other at an elevated (95° to 100° C.) temperature, by the interaction of amalgamated aluminum with distilled water. The filtered hydrous oxide was allowed to stand for a few days, then pulverized, and finally dried to constant weight in air at 25° C.

The precision of the volumetric method is excellent, and the values obtained are in very good agreement with those given by the standard gravimetric ignition procedure.

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Colorimetric Determination of Cobalt in Metallurgical Products with Nitroso R Salt

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THE routine determination of small quantities of cobalt in metallurgical products by the standard α-nitroso-β-naphthol gravimetric method is unsatisfactory. For materials ranging in cobalt content from 0.005 to 0.2%, the large initial sample and the resulting separations involving several reprecipitations render the gravimetric procedure exceedingly time-consuming and subject to the errors inherent in all lengthy analytical manipulations.

A colorimetric procedure based on the red color formed by nitroso R salt in the presence of cobalt under certain conditions has been used in recent years for determining very small quantities of this element in biological materials (*3*). Haywood and Wood (*1*) have described a photometric method for cobalt in steels, using nitroso R salt. These procedures are based on the fact that the colored complexes formed by most of the common elements with nitroso R salt, except cobalt, are destroyed by nitric acid. The full development of the color with cobalt is attained in the presence of sodium acetate, and such variables as quantity of nitric acid and of nitroso R salt, time of boiling, etc., have an influence on the development of the color.

In this laboratory several important modifications have been introduced to suit the requirements of complex ores and metallurgical products, to widen the applicability of the previous methods, and to save time and material. While the details of this procedure were worked out for final color measurements in a Spekker photoelectric absorptiometer, any other colorimetric means of determining concentrations in solutions, by photoelectric or visual colorimeters or Nessler tubes, can be employed.

PROCEDURE

REAGENTS. Cobalt sulfate, 0.2385 gram of cobalt sulfate heptahydrate dissolved and made up to 1 liter with water.

Nitroso R salt, 1 gram dissolved in water and made up to 500 ml.

Spekker acid, 150 ml. of phosphoric acid (sp. gr. 1.75) and 150 ml. of sulfuric acid (sp. gr. 1.84) made up to 1 liter with water.

Sodium acetate, 500 grams of sodium acetate trihydrate dissolved and made up to 1 liter with water.

CALIBRATION GRAPH. To 0, 1, 2, 3, etc., up to 11 ml. of standard cobalt sulfate solution are added 2 ml. of Spekker acid, 10 ml. of nitroso R salt solution, and 10 ml. of sodium acetate solution. A blank is made by adding 2 ml. of Spekker acid and 10 ml. of sodium acetate solution but omitting the nitroso R salt. The blank and the standard solutions are brought to the boil on a hot plate, 5 ml. of nitric acid are added, and the solutions are boiled for at least 1 minute and not more than 2 minutes. The standards and blank are then cooled and diluted to 100 ml. with water.

The absorption of the standards is measured on the Spekker photoelectric absorptiometer using 1-cm. cells and Hilger blue filters No. 6. The blank is set on 1.0 and the differences obtained are plotted against the weights of cobalt taken.

Table I. Comparison of Analytical Procedures

Sample	Standard Gravimetric	Nitroso R salt Operator A	Nitroso R salt Operator B
	% Co	% Co	% Co
Mill feed	0.14	0.136	0.134
Mill tail	0.06	0.056	0.062
Oxide ore	0.55	0.55	0.55
Cobalt reverberatory slag	0.63	0.66	0.62
Copper concentrate	0.64	0.63	0.60
Copper reverberatory slag	1.47	1.45	1.40
Copper reverberatory matte	1.86	....	1.80
Blister copper	0.0104	0.0102	...
Refined copper	0.0001	0.0001	...

Determination. For samples containing 0.01 to 0.20% of cobalt use a 0.25-gram sample. With higher grade materials take a 0.5-gram sample and after decomposition dilute to volume and take a suitable aliquot containing 0.01 to 0.5 mg. of cobalt for analysis. For very low cobalt contents, as in refined metals with few impurities, larger aliquots representing 1 to 20 grams may be taken. The maximum quantity of cobalt permissible in a sample is 0.5 mg., as above this concentration the solution no longer appears to obey Beer's law.

Decompose with nitric acid, hydrochloric acid, and bromine, add 5 to 7.5 ml. of 1 to 1 sulfuric acid, and evaporate to strong fumes. Some products will require the use of hydrofluoric acid for complete decomposition. Care should be taken in the addition of reagents, as an excessive concentration of salts in the final solution will increase the absorption.

Cool, dilute to about 30 ml., add 2 ml. of hydrochloric acid, and boil to solution of soluble salts. Pass a brisk current of hydrogen sulfide through the solution for 10 minutes and filter through a Whatman No. 40 filter paper, washing well with acidulated hydrogen sulfide wash water. Boil off hydrogen sulfide, add 5 ml. of nitric acid, and take to fumes of sulfur trioxide. For products like blister or refined copper, determine cobalt on the solution obtained after the electrolysis of copper. Evaporate this solution to fumes of sulfur trioxide, and continue heating until only a small amount of sulfuric acid remains.

Cool, dilute to about 25 ml. with water, and boil to solution of salts. Cool and carefully neutralize with 20% sodium hydroxide to a point where the solution becomes a deep wine red. Should there be little or no iron present, add 2 drops of phenolphthalein and take to the first faint pink. Immediately add 2 ml. of Spekker acid, then 10 ml. of nitroso R salt solution and 10 ml. of sodium acetate solution. Bring to a vigorous boil, add 5 ml. of nitric acid, and boil for at least 1 minute but not more than 2 minutes. Cool and dilute to 100 ml. with water.

At the same time carry out a blank exactly as was done in calibrating the graph. Measure the absorption of the sample in the same manner as were the standards with the blank setting at 1.0. The drum reading obtained for the sample is subtracted from 1.0 and this difference is measured off on the calibration graph to obtain the weight of cobalt present.

RESULTS

Table I compares results obtained by this procedure with those secured by careful work with standard gravimetric methods on specially prepared samples of typical metallurgical products.

For mill feed and tail 0.25-gram samples were used directly; for oxide ore, cobalt reverberatory slag, and copper concentrate, 0.5-gram samples were taken, diluted, and aliquots equivalent to 0.05 gram of original sample were withdrawn. In copper reverberatory slag and copper reverberatory matte 0.5-gram samples and aliquots equivalent to 0.025 gram were taken.

To thirteen 0.25-gram portions of a complex ore containing 4.95% copper and 3.00% iron, which was known to contain very little cobalt, varying quantities of standard cobalt solution were added. These together with another 0.25-gram portion of the ore to which no cobalt had been added were analyzed according to the foregoing procedure. Table II gives the results obtained.

DISCUSSION

Interfering Elements. It was found that even small quantities of copper prevented or masked the full development of the red cobalt complex. This confirms observations made by previous workers on biological materials (*3*), but not brought out by Haywood and Wood working with steels.

As it was necessary to remove copper and all the products on which the authors worked contained this element, a separation with hydrogen sulfide was made on all samples. With this modification, taking the usual precautions, silver, mercury, lead, copper, bismuth, cadmium, arsenic, antimony, tin, molybdenum, selenium, tellurium, gold, platinum, and palladium are removed. To 0.25-gram portions of a sample of known cobalt content (0.18%) the following elements were added as soluble salts: iron, aluminum, zinc, titanium, thorium, uranium, zirconium, tungsten, barium, strontium, calcium, and magnesium. The quantities added were sufficient to give at least 20% of each element relative to the sample; in the case of iron, enough ferric chloride was added to bring the iron content to 50%.

Cobalt was determined as usual and in no case was there observed any significant difference in cobalt recovery. Naturally some of the added elements were removed during the course of the analysis. Barium, for instance, would all be removed as sulfate. Titanium and thorium were troublesome, as hydrolyzed salts continued to be precipitated in the solution after the color had been developed. Filtration through a dry filter paper at this stage removed the turbidity and the cobalt recovery was not affected.

As no pure vanadium salt was available, a vanadium-bearing ore free of cobalt was added to a known cobalt sample, the vanadium content of the final sample being 5%. The cobalt recovery was unaffected. To the same sample varying quantities of manganese were added; up to 10% manganese there was no interference, but quantities above this had to be removed. Likewise chromium and nickel were found to be without effect up to 3 and 5%, respectively, but above these concentrations removal is indicated.

The addition of large quantities of certain of the above elements necessitated slight alterations in procedure which are commented upon in the subsequent sections.

Table II. Recovery of Added Cobalt in Ores

Sample No.	Cobalt Added	Cobalt Found	Recovery
	Mg.	*Mg.*	%
1	Nil	0.01	...
2	0.02	0.03	100
3	0.05	0.06	100
4	0.10	0.11	100
5	0.16	0.165	103
6	0.21	0.225	102
7	0.25	0.26	100
8	0.30	0.315	101
9	0.34	0.35	100
10	0.37	0.385	101
11	0.40	0.41	100
12	0.44	0.45	100
13	0.49	0.50	100
14	0.53	0.54	100

Effect of Variables in Procedure. Like all colorimetric procedures it is desirable that conditions be standardized as far as practicable and the quantities and directions given in the procedure should be adhered to as closely as possible.

Spekker Acid. Haywood and Wood used Spekker acid for the decomposition of steels. Since their reasons for using this acid did not apply to the authors' procedure, they tried dilute sulfuric acid to acidify the sample after neutralization with sodium hydroxide. They found, however, that when much iron was present low results were obtained, owing to the development of a decided brown tinge in the solution. As phosphoric acid decolorizes iron, an attempt was made to use this acid alone, but it proved unsatisfactory for most metallurgical samples. The familiar combination of phosphoric and sulfuric acids known as Spekker acid was finally tried and found successful. Varying quantities of this acid were added to known ores and to synthetic solutions. It was found that the Spekker acid could be varied from 0.5 to 3.0 ml. without affecting the color development. Any addition above 3 ml. caused a lessening of the depth of color.

Nitroso R Salt. The measurement of nitroso R salt should be accurate, but no appreciable difference was observed with quantities of nitroso R salt varying between 10 and 15 ml. The result was low with less than 10 and high with more than 15 ml. of nitroso R salt solution. The solution is perfectly stable after

standing 2 days, but it is probably advisable to make up only sufficient for the day's requirements.

Sodium Acetate. With local ores the essential minimum was 5 ml. of sodium acetate solution. The quantity of sodium acetate necessary would vary with different products and it was decided that a safe addition was 10 ml.

Nitric Acid. It is essential to add sufficient nitric acid to dissolve any precipitate formed with sodium acetate and to destroy color complexes formed with metals other than cobalt. It was found that the nitric acid could be increased to 15 ml. without affecting the color development, when 10 ml. of sodium acetate were used. The presence of certain elements necessitated alterations in procedure. Very high iron, manganese, etc., required more than the standard addition of 5 ml. of nitric acid to clear the precipitate formed with sodium acetate.

Time of Boiling. The final boiling had to be carefully controlled. The solution must be brought to a vigorous boil, nitric acid added, and the boiling continued for from 1 to 2 minutes. The extension of the boiling time to 3 minutes caused little change, but any further extension resulted in a definite bleaching of the cobalt color. On the other hand, any decrease in boiling time prevented the full development of the red color. This confirms the work of previous investigators (*1*).

Fading of Color on Exposure to Light. Samples permitted to stand for 24 hours in Erlenmeyer flasks fitted with loose covers were practically unchanged. On standing for a further 24 hours there was an observable lessening of color. The flasks were exposed to diffused sunlight for half of this period. The effect of exposure to light, although slight, is apparently more pronounced than experienced by Haywood and Wood. This is probably due to the more intense sunlight prevailing in Africa.

It would appear that when dealing with quantities of cobalt between 0.01 and 0.50 mg. the control of conditions need not be so critical as described by Kidson and Askew (*2*), who were working with quantities of cobalt as low as 0.0001 mg. While Haywood and Wood (*1*) report good results with very high cobalt-containing steels, the authors have found the greatest range of usefulness of this method to lie in the lower concentrations of cobalt in mining and metallurgical products.

SUMMARY

A modification of the nitroso R salt method for cobalt is described which should have wide adaptability for the determination of small quantities of this element in metallurgical products and similar materials. Results compare very favorably in accuracy and precision with those obtained by the longer standard gravimetric procedures, and the recovery of added cobalt is complete.

A comprehensive review of the effect of other elements and of varied conditions is given. The determination is best carried out on a sample or aliquot containing 0.01 to 0.5 mg. of cobalt. Apart from the elements removed with hydrogen sulfide in the course of the determination, this work to date shows that the final color comparison can be made in the presence of at least 1000 times as much iron, 100 times as much aluminum, zinc, titanium, thorium, uranium, zirconium, tungsten, barium, strontium, calcium, and magnesium, and 25 times as much vanadium. Quantities of manganese in excess of 50 times, nickel beyond 25 times, and chromium in excess of 15-fold the cobalt present, interfere and must be removed.

Although details are presented for the final determination of cobalt in the Spekker absorptiometer, the method is adapted for any other means of photoelectric or visual color comparison.

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Radioactive Studies

Utilization of the Radioactive Isotope Dilution Procedures for Special Types of Chemical Problems. Quantitative Determination of the Three Individual Components of Mixtures of Dibenzyl Sulfide, Sulfoxide, and Sulfone as an Illustrative Example[1]

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ALTHOUGH certain highly specialized instruments, such as spectrometers (infrared, mass, etc.) and polarographs enable the analysis of certain individual components of many multicomponent systems, there are still numerous types of mixtures that cannot be quantitatively analyzed by means of these apparatus.

It has been known for some time that the isotope dilution method is applicable in principle to any analytical problem (*6*). Recently Foster, Rittenberg, and others (*1*, *2*, *7*) have begun to use stable isotopes in the determination of the amino acid content of proteins. In the case of radioactive isotopes, the usual analytical errors of 10 to 15% prevented their usage in the isotope dilution method (*9*). Since recent studies (*3*, *4*) have shown that some of the radioactive tracers most difficult to quantitate—namely, long-lived sulfur, arsenic, carbon, and hydrogen—can be analyzed to within 2%, the accuracies of the isotope dilution method, with either stable or radioactive isotopes, are essentially identical. This communication indicates the full scope of the radioactive isotope dilution method as applied to chemical problems and gives a specific experimental example, using S^{35} of 87-day half-life.

[1] This is the fifth in a series of articles on Radioactive Studies. Previous articles are found in June and July issues.

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GENERAL CONSIDERATIONS

Let P be a nonradioactive compound. Let P^* be the same compound in which a minute fraction of the molecules contain a tracer element in a stable position, and let the radioactivity per milligram of this compound be denoted by $(act/mg)_{P^*}$. Let $P.P^*$ denote the chemically indistinguishable mixture of P and P^*, and let the radioactivity per milligram of this mixture be denoted by $(act/mg)_{P.P^*}$. Let the ratio of the specific activities $(act/mg)_{P^*}/(act/mg)_{P.P^*}$, be defined as R.

There are two general procedures in applying the isotope dilution method.

PROCEDURE I. The amount of P in a multicomponent system is unknown. If a known quantity of P^* is added to and dispersed uniformly throughout the mixture, the amount of P can be determined, provided that a weighable aliquot ($\geq$0.05 mg.) of $P.P^*$

ne radioactive isotope dilution method is a powerful tool which applicable to analytical problems that cannot be solved readily y any other means. There are two general variations to this method; ne is best suited to the analysis of the individual constituents of ulticomponent systems, and the other is useful in the investigation f mechanisms and/or yields of reactions. As an illustrative example ilizing long-lived radioactive sulfur, S^{35}, quantitative data are esented for the analysis of the three individual components of ixtures of dibenzyl sulfide, sulfoxide, and sulfone. These data ow that these analyses, if done in triplicate, can be made with n average error of less than 1% and a maximum error of less than 2%.

an be isolated in a pure state. This relationship is given by Equation 1:

$$mg_P = mg_{P^*}\,(R - 1) \qquad (1)$$

Procedure II. The amount of P^* in a multicomponent system containing other radioactive compounds is unknown. The addition of a predetermined amount of P sufficient to isolate subsequently a weighable quantity of $P.P^*$ in a pure state, followed by complete mixing of the active and inactive species, will enable the analysis to be made. Equation 2 gives this relationship:

$$mg_{P^*} = mg_P\,[1/(R - 1)] \qquad (2)$$

For both Procedures I and II, the specific activity of P^* can be predetermined to make $R \gg 1$ and to make each radioactive measurement with a maximum error of 2%; thus the maximum uncertainty of an individual analysis by the radioactive isotope dilution method is 4%.

Procedure I is best suited for any problem involving the analysis for the individual components of a multicomponent system, provided that sufficient P is present to enable the isolation of a weighable amount of PP^*. Thus it cannot be used for the determination of traces of substances.

Procedure II is useful in the investigation of the mechanism and/or yield of reactions. This is most readily shown by considering a specific example—namely, the following hypothetical polymerization:

$$A + P = A_1P + A_2P + A_3P + \ldots = \sum_1^N A_KP \qquad (3)$$

where A polymerizes in the presence of the catalyst, P, to A_K and the polymerization is terminated by the addition of P to the polymer. It is essential that the polymers, A_KP, can be obtained in pure form—e.g., be reacting large quantities of A and P and subsequent fractional distillation and/or crystallization. Then the synthesis of P^* and the use of this compound instead of P in reaction 3 enables the determination of the exact amount of each A_KP^* formed, since $(act/mg)_{A_KP^*}$ can be computed stoichiometrically from $(act/mg)_{P^*}$, and $(act/mg)_{A_KP \cdot A_KP^*}$ is experimentally determined.

Methods similar to those outlined above are applicable to any reaction. Unlike Procedure I, Procedure II enables the analysis of traces of substances; in fact, since most radioactive isotopes can be obtained in high millicurie strength, it is possible to analyze quantitatively for less than 0.001 microgram of many compounds—e.g., A_KP^*—by the isotope dilution method. It is here that radioactive isotopes come into their own, since the allowable dilution factors are much greater than those of stable isotopes.

Utilizing radioactive sulfur, S^{35}, the authors have used both Procedures I and II; the former is considered in the next section and the latter has been used to investigate the mechanism of mustard gas vesication (*5*). The procedures are interdependent and the accuracy to be expected from either isotope dilution method is essentially identical.

ILLUSTRATIVE EXPERIMENTAL EXAMPLE

In order to evaluate the radioactive isotope dilution method as a quantitative analytical procedure, an illustrative example of Procedure I will be considered in detail—the analysis of the three individual components of mixtures of dibenzyl sulfide, sulfoxide, and sulfone. In essence the procedure consists of the following steps for this particular case:

Dibenzyl sulfide, sulfoxide, and sulfone, each containing radioactive sulfur, are synthesized.

The amount of radioactivity per milligram, $(act/mg)_{P^*}$, is determined for each radioactive compound.

Accurately weighed amounts of each of the radioactive compounds are added to the unknown mixture of dibenzyl sulfide, sulfoxide, and sulfone.

A portion of each substance is isolated in the pure state by fractional crystallizations.

Weighed amounts of the three purified fractions are subjected to radioactive analysis and the $(act/mg)_{P.P^*}$ is computed for each substance. From the reduction in specific activity from $(act/mg)_{P^*}$ to $(act/mg)_{P.P^*}$ together with the known weight of radioactive compound added, the amount of each of the original compounds present in the unknown mixture is calculable by means of Equation 1.

Preparation of the Compounds. About 20-gram quantities of nonradioactive dibenzyl sulfide, sulfoxide, and sulfone were prepared by the method described in detail by Shriner *et al.* (*8*).

This procedure is based on the following schematic reactions:

$$C_6H_6CH_2Cl + Na_2S \longrightarrow (C_6H_6CH_2)_2S \qquad (4)$$

$$(C_6H_6CH_2)_2S + H_2O_2 \longrightarrow (C_6H_6CH_2)_2SO \qquad (5)$$

$$(C_6H_6CH_2)_2SO + CrO_3 \longrightarrow (C_6H_6CH_2)_2SO_2 \qquad (6)$$

Since the techniques involved in the preparation of dibenzylsulfide on the milligram scale are rather different, the preparation of this compound containing radioactive sulfur is described in detail. The radioactive sulfur used in this preparation was obtained from the Massachusetts Institute of Technology, Radioactivity Center.

Dry barium sulfate (200 mg.) containing about 30 microcuries of S^{35} (87-day half-life) was placed in a platinum boat which was introduced into a long quartz tube. By heating the tube to 800° C. for 4 hours, while passing through a stream of hydrogen, a 98% reduction to barium sulfide was obtained. The trace of hydrogen sulfide formed by the side reaction

$$BaSO_4 + 4H_2 \longrightarrow BaO + H_2S + 3H_2O \qquad (7)$$

was caught in a zinc chloride trap. The resulting zinc sulfide precipitate was added to the barium sulfide in the platinum boat.

The boat with contents was transferred to a small gas generator containing 1 gram of 20-mesh zinc. Sufficient 6 *M* phosphoric acid to cover the boat was now added through a stopcock and the mixture was heated. The issuing gases were dried with phosphorus pentoxide, and the hydrogen sulfide was condensed in a trap chilled by liquid nitrogen. The purpose of the hydrogen was to sweep out all traces of hydrogen sulfide from the generator with a noncondensable gas. The hydrogen sulfide was then stored in an evacuated bulb attached to a vacuum system.

A tube, containing 1.85 millimoles (235 mg.) of benzyl chloride 1.85 millimoles (75 mg.) of sodium hydroxide, 6 cc. of ethyl alcohol, and 1 cc. of water, was attached to the vacuum system by means of a ground joint and frozen down and evacuated. The hydrogen sulfide (0.85 millimole) was vacuum-distilled into this tube chilled with liquid nitrogen. This tube was then sealed and placed on a steam bath for 3 days. The tube was opened and the contents were extracted four times with petroleum ether (b.p. 40° to 60° C.). The petroleum ether solution was placed in a test tube, and the volatile contents were pumped away; the contents of the apparatus were further pumped for 1.5 hours to remove most of the residual benzyl alcohol and benzyl chloride. The dibenzyl sulfide was recrystallized from ethyl alcohol.

Yield, 168 mg., 92% based on barium sulfate; m.p. 49° C.

To prepare the dibenzyl sulfoxide, 120 mg. of radioactive dibenzyl sulfide were used.

Yield, 107 mg., 83%; m.p. 134° C.

To prepare the sulfone, 60 mg. of the radioactive sulfoxide were used.

Yield, 45 mg., 70%; m.p. 149° C.

For these last two compounds, Shriner's method (*8*) scaled down to these quantities was exactly followed.

Thus, about 45 mg. of each compound containing about 10 microcuries of S^{35} were prepared. This quantity of material would allow the analysis in triplicate of at least 100 unknown mixtures of the three dibenzyl compounds.

Table I. Triplicate Radioactive Sulfur Standardizations of the Three Dibenzyl Compounds

[a]	$(C_6H_5CH_2)_2S^*$	$(C_6H_5CH_2)_2S^*O$	$(C_6H_5CH_2)_2S^*O_2$
$act/mg)P^*$	169.0	156.5	148.7
	169.6	160.0	148.1
	169.7	158.8	147.2
Av. $(act/mg)P^*$	169.4	158.4	148.0
Av. $(act/micromole)P^*$	36.2	36.4	36.4

[a] The activity of each sample is computed by the following formula:

$$\text{Activity} = [(d/t)_s - (d/t)_b] \times \frac{10^2}{f} \qquad (8)$$

where d is the number (10) of scale divisions traversed by the electroscope fiber in time t in seconds; s and b denote sample and background, respectively; and f is the beta-ray self-absorption correction factor due to the weight of the benzidine sulfate precipitate (see *3* for further details).

With the electroscope used, one unit of activity represents about 10^{-3} microcurie of S^{35}.

Table II. Analysis of Known Mixtures of Dibenzyl Sulfide, Sulfoxide, and Sulfone by Radioactive (S^{35}) Isotope Dilution Procedure

	Mixture of Nonradioactive Compounds								
	$(C_6H_5CH_2)_2S$			$(C_6H_5CH_2)_2SO$			$(C_6H_5CH_2)_2SO_2$		
Mixture No.	Weight added	Weight determined by S^{35} analysis		Weight added	Weight determined by S^{35} analysis		Weight added	Weight determined by S^{35} analysis	
	Mg.	*Mg.*		*Mg.*	*Mg.*		*Mg.*	*Mg.*	
		Av.	Individual analysis		Av.	Individual analysis		Av.	Individual analysis
1	400	406	412 395 410	400	399	403 401 392	400	399	409 390 397
2	400	402	396 403 406	400	399	403 395 398	400	397	393 406 393
3	400	396	389 400 399	400	401	392 404 406	400	395	397 401 390
4	200	199	203 197 196	400	400	404 391 404	1400	138_0	1355 1402 1390
5	400	400	399 400 402	1400	139_0	1368 1408 1396	200	203	205 199 206
6	1400	140_0	1382 1415 1404	200	201	206 193 204	400	407	405 406 410

Radioactive Standardizations. Approximately 1 mg. of each of the three radioactive compounds was carefully weighed out on a microbalance and oxidized to the sulfate ion by the Carius method. Each sample was washed into its respective 100-cc. volumetric flask. Triplicate 3-cc. aliquots were then subjected to S^{35} analyses by the procedure described elsewhere in detail (*3*).

Table I shows the reproducibility of these radioactive standardizations as measured by the modified Lauritzen electroscope.

These data show that with care accurate radioactive sulfur analysis can be made. They also are indicative of the purity of the radioactive compounds.

Preparation of Radioactive Standard Solution. About 15 mg. of the radioactive sulfide, sulfoxide, and sulfone were weighed out, respectively, to within 0.05 mg. This mixture was placed in a volumetric flask and made up to 100 cc. with 95% ethyl alcohol. Thus a known volume of this solution contained a predetermined weight of each of the three radioactive compounds. As an additional check, triplicate radioactive analyses were made on 1-cc. aliquots of this solution. The average of the activities obtained was within 0.4% of that computed from the radioactive standardizations of the individual components.

Isolation of Pure Portions of Each Compound ($P.P^*$) for Radioactive Analysis. To an acetone solution containing the mixture, P, of nonradioactive dibenzyl sulfide, sulfoxide, and sulfone to be analyzed, exactly 10 cc. of the radioactive standard solution, P^*, were added. The solution was well stirred and the acetone and alcohol were evaporated off. The solid mixture was now ready for the isolation of the pure portions of the three compounds, $P.P^*$. The procedure used was based on the relative solubility of these compounds in petroleum ether (b.p. 40° to 60° C.) and 95% ethyl alcohol.

Cold petroleum ether was added; the sulfide was partially separated by filtering, since the sulfoxide and sulfone were somewhat insoluble. The remaining solid was dissolved in alcohol upon cooling, some of the sulfone precipitated while most of the sulfoxide remained in solution. Thus, there now remained a petroleum ether solution of crude sulfide, alcoholic solution of impure sulfoxide, and solid crude sulfone. The petroleum ether and alcohol were respectively evaporated off and the three impure solids were recrystallized three times, the sulfide from 95% ethyl alcohol, the sulfoxide from petroleum ether (b.p. 40° to 60° C.), and the sulfone from 95% alcohol. The melting point of each compound was taken, and if it were not correct further recrystallizations were made.

Six different mixtures containing various known amounts of the three dibenzyl compounds were subjected to the above procedure. Since 200 mg. was the minimum amount of any of the dibenzyl compounds, P, in the original mixture, and 20 mg. more than sufficed for radioactive analysis, it was not difficult to obtain the three substances in a highly purified state. Triplicate radioactive sulfur analyses (*3*) were made on weighed amounts (~6 mg.) of each purified component, $P.P^*$, and the radioactivities were calculated by means of Equation 8.

The experimental results, as computed by Equation 1, are given in Table II. In order to indicate the magnitude of the experimental error, each individual determination has been included in Table II. The greatest deviation found between the amount of dibenzyl compound added and an individual analysis is 3.2% (1400 mg. of dibenzylsulfone added, 1355 mg. found; mixture 4). The average per cent deviation between an individual analysis and the amount of compound added is 1.4. The maximum and average per cent deviation between the average of the triplicate analyses and the weight of dibenzyl compound added are 1.8 and 0.7, respectively.

Thus the accuracy of these data compares favorably with that obtained by using stable isotopes in isotope dilution Procedure II (*2*). It is believed that with care this error of less than 2% could be duplicated in all analyses, utilizing either radioactive isotope dilution Procedure I or II. Since there are numerous analytical problems that cannot be solved readily in any other manner, the radioactive isotope dilution method will ultimately take its place among the other highly specialized analytical techniques.

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Analytical Chemistry of the Rare Earths

The Active-Oxygen Determination

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A method is described for the measurement of the active oxygen present in a mixture of ignited rare earth oxides. The procedure is superior to the classical Bunsen method in that no difficulty is encountered in the analysis of high ceria mixtures. Data are given for the analysis of pure cerium dioxide, of some cerium dioxide–lanthanum sesquioxide mixtures, and of pure praseodymium oxide.

IN GENERAL, gravimetric methods of analysis are based upon a separation followed by a final weighing of the desired constituent in a pure compound of known composition. However, in the analysis of rare earth materials, separation of the individual earths is impractical and one is faced with the necessity of weighing a mixture which may contain elements of the entire group. Certainly the oxalates of cerium, praseodymium, and terbium yield upon ignition in air oxides containing more oxygen than can be accounted for by sesquioxide formation (*1*), and there is some indication of a higher oxide of neodymium (*6*). The situation is further complicated by the possible influence of certain other rare earths upon the higher oxides formed by the elements mentioned (*2, 3, 5*).

The evaluation of the true rare earth metal content of a mixture of such oxides would be greatly simplified by a method which permitted direct measurement of the so-called "active oxygen", the oxygen present in excess of that in the sesquioxide. Correction of the weight of the oxide mixture would then permit calculation of an accurate metal content.

Such an analytical method would be useful also in obtaining data regarding the composition of rare earth oxides formed under various conditions, both in the pure state and in mixtures. Such studies have been made in this laboratory using the method described below and will form the subject of a later paper.

The method of Bunsen (*4*) has found wide application for the determination of higher-valent cations in compounds similar to the rare earth metal oxides. Hydrochloric acid is added to the compound under investigation and the chlorine liberated by the reaction is absorbed in a potassium iodide solution. The liberated iodine is then titrated with thiosulfate solution in the usual manner.

Such a procedure is not feasible, however, if an appreciable amount of cerium is present in the mixture under investigation. Cerium dioxide, once formed, is extremely inert to all but the strongest reducing agents and even extended contact with concentrated hydrochloric acid results in very little attack.

EXPERIMENTAL

Several modifications of the Bunsen method were attempted, using both arsenious oxide and sodium oxalate as reducing agents. The reaction rate between the oxides proved to be too slow to be useful, while the conditions necessary to complete the reaction with sodium oxalate were so vigorous that appreciable decomposition of the oxalate in the sulfuric acid medium occurred.

A simple procedure using potassium iodide and a modified Bunsen apparatus was finally developed which gave satisfactory results.

The apparatus consisted of a 125-ml. Kjeldahl flask fitted through a ground-glass joint with a water-cooled reflux condenser. To the upper end of the condenser was attached, by a ground-glass joint, a tube bent to a semicircle and terminated with a Bunsen valve. When in use, the valve was immersed in a potassium iodide solution contained in a small beaker.

An accurately weighed sample of the pure oxide or mixture of oxides is placed in the Kjeldahl flask and 20 ml. of a 10% potassium iodide solution are added. Eighty milliliters of a 4% solution of potassium iodide are placed in the beaker to cover the Bunsen valve.

Ten milliliters of 12 *N* hydrochloric acid are added to the flask, the flask is attached immediately to the reflux condenser, and the mixture is heated gently until dissolution of the oxide is complete. The flask is cooled, the vapor trap removed, and any sublimed iodine clinging to the tube of the condenser is washed into the Kjeldahl flask with the vapor-trap solution.

The solution is then transferred to a 500-ml. Erlenmeyer flask, diluted to 350 ml., and titrated to a starch end point with standard 0.1 *N* sodium thiosulfate solution.

A blank is determined under conditions identical to those in the analysis, using similar periods of heating and volumes of solution. The blank in no case should exceed 0.5 ml. The difference between the two volumes is, of course, equivalent to the iodine released by the active oxygen present in the sample.

$$\text{Active oxygen (mg.)} = (N \text{ of } Na_2S_2O_3)\ (\text{ml. of } Na_2S_2O_3)\ (\text{meq. of } O_2) \quad (1)$$

Values obtained by the analysis of samples of pure cerium dioxide with or without admixture of pure lanthanum sesquioxide and using the method described are shown in Table I.

Table I. Active Oxygen in Pure Cerium Dioxide and in Cerium Dioxide–Lanthanum Sesquioxide Mixtures

Weight of Sample	La_2O_3	Active Oxygen Taken	Active Oxygen Found
Mg.	%	*Mg.*	*Mg.*
194.7	...	9.0	9.0
285.6	...	13.2	13.3
202.5	4.81	8.9	8.9
237.2	4.81	10.5	10.4
274.0	25.49	9.5	9.5
189.2	25.49	6.6	6.6
197.1	49.37	4.6	4.7
170.8	49.37	4.0	4.0
269.1	72.91	3.4	3.4
271.2	72.91	3.4	3.5
541.8	93.88	1.5	1.6
394.6	93.88	1.1	1.2

COMPOSITION OF PRASEODYMIUM OXIDE

The application of the method is well illustrated by a study of the ignition product of pure praseodymium oxalate.

The material used in this investigation was purchased from Adam Hilger, Ltd., 98 Kings Road, London, England, and bears the laboratory number 6782. It was obtained by Hilger from Professor Rolla at the University of Florence. Independent spectrographic analyses by the Hilger laboratory and by V. W. Prandtl show impurities only of magnesium (0.1%) and dysprosium (0.02%).

The pure oxalate was precipitated from dilute nitric acid in the usual manner and, after oven-drying, was ignited to constant weight at 955° ± 10° C. After cooling, weighed portions of the ignition product were analyzed as described above. Table II illustrates the results obtained.

Table II. Active Oxygen in Pure Praseodymium Oxide

Weight of Sample	Volume of 0.0459 *N* $Na_2S_2O_3$	Active Oxygen		
Mg.	*Ml.*	*Mg.*	%	Fraction
187.4	16.47	5.98	3.19	0.0319
143.2	12.57	4.61	3.22	0.0322
155.5	13.20	4.94	3.18	0.0318

[1] Present address, Magnolia Petroleum Co., Dallas, Texas.

Now, representing the general formula of the oxide obtained as Pr_xO_y, the ratio of y/x may be calculated by the relation

$$\frac{x\,(\text{atomic weight of praseodymium}) + y\,(\text{atomic weight of oxygen})}{\frac{x}{2}\,(\text{formula weight of } Pr_2O_3)} =$$

$$\frac{140.92\,x + 16.00\,y}{\frac{x}{2}\,(329.84)} = \frac{1}{1 - \text{fraction of active oxygen}}$$

From the data of Table II, using the average value 0.032 for the fraction of active oxygen, the ratio of y/x is calculated to be 1.84, a value corresponding to the following formulas:

$$Pr_4O_{7.36},\ Pr_5O_{9.20},\ Pr_6O_{11.04},\ Pr_7O_{12.88}$$

From the data presented, it appears that the product of the ignition of pure praseodymium oxalate in air at 955° C. has a composition most closely approximating the formula Pr_6O_{11}. Ignition at temperatures of 670° and 780° C. had little effect upon the fraction of active oxygen in pure praseodymium oxide, the values obtained in these cases being 0.031.

SUMMARY

A procedure for the determination of active oxygen in mixtures resulting from the ignition of mixed rare earth oxalates is particularly applicable to mixtures containing a high percentage of ceria. Additional proof of the composition Pr_6O_{11} for the oxide formed upon the ignition of pure praseodymium oxalate at various temperatures has been obtained.

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Estimation of Ammonium Picrate in Wastes from Bomb- and Shell-Loading Plants

Some Reactions of Ammonium Picrate in Water and Sewage

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A practical spectrophotometric procedure for determining the ammonium picrate content of wastes or the ammonium picrate, alpha-TNT, and colored TNT in wastes containing all three is presented. The procedure, which involves extinction readings at 460 and 505 mμ after three steps of treatment of the sample, is simple and rapid. Ammonium picrate in wastes exhibits no biochemical oxygen demand and in concentrations above 300 p.p.m. inhibits the normal B.O.D. reaction of polluted water. Taste tests on 20 people indicated that concentrations of 1.0 p.p.m. or more of ammonium picrate are objectionable in drinking water.

IN CONNECTION with the study of wastes from U. S. Naval Ammunition Depots, the estimation of ammonium picrate wastes in streams and in waters containing TNT wastes was necessary. It was also desirable to determine the effects of ammonium picrate in such wastes on sewage disposal plants and other disposal methods, as little is known about the fate of such wastes or their effects on natural biological purification.

QUALITATIVE TESTS FOR AMMONIUM PICRATE

Several qualitative and quantitative methods were investigated before the spectrophotometric method was adopted. The following tests were applied to ammonium picrate wastes, with the results noted.

1. The ammonium sulfide reagent (*4*) is insensitive to ammonium picrate solutions under 100 p.p.m.
2. The potassium thiocyanate test (*6*) is sensitive at concentrations of ammonium picrate as low as 5 p.p.m. However, on alkalizing the solution, a brown color due to the presence of alpha-TNT makes this method unsatisfactory for most cases.
3. The methylene blue test (*1*) is very sensitive to ammonium picrate if the precipitate obtained is extracted with chloroform, the shade of color obtained being dependent upon the pH of th water. As 5 p.p.m. of TNT interfere with this test, it is cor sidered less satisfactory than the test with sodium hydrosulfit which is described below.
4. According to Davis (*3*) guanidine picrate is much les soluble than ammonium picrate. However, laboratory exper ments showed that a quantitative precipitation was not obtaine on concentrations of ammonium picrate below 100 p.p.m Therefore, this test is too insensitive for normal ammonium pi rate wastes.
5. The reaction with sodium hydrosulfite (*2*) and ammoniu picrate, forming the red picramate, is sensitive to 0.5 p.p.m. ammonium picrate. TNT does not appreciably interfere wit this reaction. The color produced, however, is of short duratio (1 to 2 minutes) and must be read immediately. Following a the details for carrying out this test under optimum condition

Reagents. Sodium hydrosulfite solution, 1.0%. Dissol 1.0 gram of sodium hydrosulfite in 100 ml. of approximately 0 *N* sodium hydroxide solution.

Buffer solution, pH 7.2. Dissolve 134 grams of potassiu dihydrogen phosphate in several hundred milliliters of distille water. Add a solution of normal sodium hydroxide until a pH 7.2 is reached and make up to 1 liter.

Procedure. Add 50-ml. portions of the sample to be teste to each of two 100-ml. Erlenmeyer flasks. If the pH of the wat is below 7.0, add 1 ml. of the 7.2 pH buffer solution to each flas Then add 1.0 ml. of the sodium hydrosulfite solution to one flas swirl the flask, and compare immediately with the untreate control. A white background will facilitate comparison. Th appearance of a momentary red intensification indicates that am monium picrate is present. With low concentrations of am monium picrate, immediate comparison is important, as the re color of ammonium picramate will fade within 2 minutes.

If a yellow intensification is noted, dilute a part of the sampl treat with sodium hydrosulfite as above, and compare with a untreated diluted portion. A slight yellow to brown more pe manent intensification indicates a large amount of alpha-TN As the sensitivity of the test is about 0.5 p.p.m., a rough quan tative estimate of the ammonium picrate in p.p.m. in the samp

Table I. Extinction Values of Ammonium Picrate Solutions

Concentrations of Ammonium Picrate, P.P.M.	Extinction at 460 Mμ: In distilled water (calculated $K = 0.033$)	In solution of 300 p.p.m. of Na_2CO_3	In distilled water plus 1 gram of Na_2CO_3 per 50 ml.
0.0	0.03	0.03	0.04
2.0	0.10	0.10	..
2.5	0.11	0.11	..
3.3	0.14	0.16	..
4.0	0.16	0.17	..
5.0	0.20	0.19	0.20
8.0	0.29	0.30	..
10.0	0.36	0.36	0.37
20.0	0.69	0.68	..

can be made by multiplying by 2 the highest dilution giving a positive test.

QUANTITATIVE DETERMINATION OF PICRATE WITH VISUAL SPECTROPHOTOMETER

After the investigation of several quantitative and qualitative methods, the spectrophotometric method, was considered most satisfactory for use on ammonium picrate wastes.

In all spectrophotometric determinations, turbidity must be eliminated from the sample. This is usually done by sedimentation, filtration, or centrifugation. If none of these methods removes the extremely fine turbidities sometimes encountered in natural waters, coagulation with alum can be applied. However, this method of clarification should be used only as a last resort, as it will remove as much as 25% of ammonium picrate or TNT in dilute samples containing up to 10 p.p.m. of these materials.

Ammonium picrate in the absence of TNT wastes can easily be determined colorimetrically or with the spectrophotometer. Although the maximum absorption occurs in the ultraviolet range at 335 mμ, the maximum absorption in the visible range can be used for practical purposes. Using dilutions from a stock 300 p.p.m. solution, an absorption curve was first plotted and it was observed that maximum absorption in the visual range occurred at 460 mμ. Extinction values for dilutions of the stock solution in distilled water and in water containing 300 p.p.m. of sodium carbonate were determined. The mean extinction values obtained are shown in Table I.

These data indicate that the concentration of carbonate or bicarbonate present in natural waters would have no effect on the extinction of ammonium picrate. The increment of E per p.p.m. of ammonium picrate is small (0.033); hence, this method of estimating ammonium picrate concentrations is less sensitive and less accurate than the similar determination for TNT (*5*). Nevertheless, concentrations of ammonium picrate between 2 and 40 p.p.m. can be estimated without dilution. Stronger solutions can be diluted before examination. On the basis of data shown in Table I, the concentration of ammonium picrate in clear solutions is obtained as follows:

$$\text{P.p.m.} = \frac{E_s - E_b}{K} \times (d)$$

where E_s = extinction of diluted sample
E_b = extinction of clear water blank
d = dilution factor
K = 0.033 = extinction per p.p.m. of ammonium picrate for the instrument in use

DIFFERENTIATION AND ESTIMATION OF AMMONIUM PICRATE AND TNT WITH VISUAL SPECTROPHOTOMETER

The method used to differentiate and determine the quantity of alpha- and colored TNT present in TNT waste with the spectrophotometer has been described (*5*). This procedure depends upon a measurement of extinction at 460 and 505 mμ on the clear colored liquid, followed by treatment of the solution by sulfite and hydroxide as described and re-examination at 460 and 505 mμ. When dilute solutions of ammonium picrate are put through this procedure for TNT determination, a color intensification is also obtained by the sulfite hydroxide treatment. In this case there is no great change in the shape of the absorption curve after sulfite treatment, for the solution continues to have its highest E values at the lowest visual wave lengths, with a rapid loss in extinction before 505 mμ is reached. However, when the sulfite hydroxide–treated sample of ammonium picrate is acidified with strong hydrochloric acid, the reaction as far as absorption is concerned is so different from that with TNT solutions that differentiation is possible. When the sulfite hydroxide–treated ammonium picrate sample is acidified, the yellow color is reduced in intensity to what it was before the sulfite hydroxide treatment. With alpha-TNT solutions, acidification accomplishes complete decolorization. Colored TNT solutions are also almost entirely decolorized by the strong acid treatment. It would seem reasonable, therefore, for practical purposes to use the color and absorption values remaining after sulfite and hydroxide treatment followed by acidification as a further step in the analysis for ammonium picrate. Acidification of the 50-ml. sample with 5 ml. of C.P. concentrated hydrochloric acid, followed by a third set of extinction readings at 460 and 505 mμ, has therefore been added to the procedure previously described for TNT wastes for analysis of shell- and bomb-loading wastes which may also contain ammonium picrate.

If the sample is turbid, it is settled and filtered through paper and/or centrifuged until clear. If a clear solution cannot be obtained, the extinction determined on the untreated sample cannot be used. If the solution obtained is free from turbidity, the color is due to ammonium picrate, colored TNT, or organic matter, or mixtures of these, and the clarified sample is examined at 460 and 505 mμ. For this examination the sample may be diluted, if necessary, in distilled water, or better in distilled water containing 300 p.p.m. of sodium carbonate. When the extinctions on the clarified sample at 460 mμ are only slightly higher than at 505 mμ, colored TNT (and possibly organic matter) rather than ammonium picrate, are indicated. If the extinction at 460 mμ is considerably higher than at 505 mμ, but finite values are observable at both wave lengths, both ammonium picrate and colored TNT are probably present. Ammonium picrate solutions in concentrations up to about 100 p.p.m. even undiluted show no extinction at 505 mμ, though high E values are given at 460 mμ. Consequently, if there is a finite extinction at 460 mμ but none at 505 mμ, only ammonium picrate is indicated.

Following the examination of the clarified sample, a 50-ml. portion is treated with sulfite and hydroxide as described for the analysis of TNT wastes; and after filtration is diluted if necessary in the special sulfite hydroxide dilution water and examined at 460 and 505 mμ. If there is a new gain in extinction at 505 mμ due to the sulfite hydroxide procedure and if the extinction after this treatment is higher at 505 mμ than at 460 mμ, the indications are that only colored and alpha-TNT are present. Under these circumstances the calculation for colored and alpha-TNT is made as has been described (*5*).

If after the sulfite hydroxide treatment the extinction at 505 mμ is higher than before this treatment, but lower than at 460 mμ after treatment, both ammonium picrate and TNT may be indicated; and the final acidification step is necessary to complete the analysis. However, if the extinctions at both wave lengths are equal or are lower after sulfite hydroxide treatment than before, it indicates that ammonium picrate is absent and either (1) that the higher original extinctions (in perfectly clear samples) were due to colored organic matter and not to TNT, or (2) that colored TNT is present and alpha-TNT absent. The second explanation of the above extinction relationships has been found to be correct on all water samples examined by the authors, and the first explanation was correct only on some sewage effluent samples.

In the case of the examination of relatively highly colored clarified sewage samples, it is best to ignore the original extinction readings and make the analysis on the basis of the qualitative hydrosulfite test for ammonium picrate and the extinction read-

Table II. Extinction Characteristics and Constant and Extinction Symbols for the Three-Step Spectrophotometric Procedure

Step Following	Clarification		Sulfite Hydroxide Treatment		Acidification	
E reading at, mμ	460	505	460	505	460	505
E characteristics						
Ammonium picrate	+	0	++	+	+	0
Colored TNT	+	±	+	±	±	±
Alpha-TNT	0	0	+	++	0	0
Symbol for E						
Observed reading	E_{OL1}	E_{OH1}	E_{OL2}	E_{OH2}	E_{OL3}	E_{OH3}
Component for ammonium picrate	E_{PL1}	0	E_{PL2}	E_{PH2}	E_{PL3}	0
Colored TNT	E_{CL1}	E_{CH1}	E_{CL2}	E_{CH2}	E_{CL3}	E_{CH3}
Alpha-TNT	0	0	E_{AL2}	E_{AH2}	0	0
Symbol for specific constant to be applied						
Ammonium picrate	K_{PL1}	...	...	K_{PH2}	K_{PL3}	...
Colored TNT	K_{CL1}	K_{CH1}	...	K_{CH2}	...	K_{CH3}
Alpha-TNT	...	...	...	K_{AH2}	...	...

\+ = finite E value. ++ = E value greater than +.
± = E value less than +. 0 = No finite E.

ings after treatment of the sample with sulfite and hydroxide, and again after acidification for alpha- and colored TNT.

If the analysis has proceeded through the sulfite hydroxide treatment and the question of the presence of ammonium picrate remains, a 50-ml. portion after sulfite hydroxide treatment is acidified with 5 ml. of concentrated C.P. hydrochloric acid. After the acid has been allowed to react for about a minute, the sample is examined again at 460 and 505 mμ. Complete decolorization—i.e., extinction readings no longer than the blank for the machine at 460 mμ—indicate that both ammonium picrate and colored TNT are absent. If, however, a decided yellow color remains and there is a considerable fall in extinction from 460 to 505 mμ, ammonium picrate is indicated and colored TNT may also be present. However, all ordinary concentrations of ammonium picrate (up to about 100 p.p.m. undiluted) show no extinction at 505 mμ, so that the E reading at 505 mμ after acidification indicates only colored TNT.

CALCULATION OF EXTINCTION DATA

To help illustrate how extinction data for this procedure can be readily interpreted and calculated to the various constituents, a system of identification of the extinction values for each wave length read and each step in the procedure is introduced.

For this purpose, let us label all observed extinctions as E_O and the calculated or extinction components for ammonium picrate, colored TNT, and alpha-TNT as E_P, E_C, and E_A, respectively. To indicate the wave length at which the readings were made, we will add H for 505 mμ and L for 460 mμ to the above symbols. Finally, to indicate whether the extinctions were read after clarification (step 1), sulfite hydroxide treatment (step 2), or acidification (step 3), let us add the figures 1, 2, and 3, respectively. To illustrate, E_{OL1} means the observed extinction at 460 mμ after the first step; and E_{AH2} means the extinction component for the alpha-TNT at 505 mμ after the second step or the sulfite hydroxide treatment. Although the E_P, E_C, and E_A values are not observed directly in a mixed sample, these extinction components must be obtained before the concentrations of these constituents can be estimated. If however, the sample contains only colored TNT, then E_{OL1} becomes also E_{CL1}.

In all cases the constant or K value must be known for each constituent at the wave length and step at which the extinction component is to be converted to the concentration of the component. These constants must be determined for each machine on a series of diluted standards for the particular component, step, and wave length needed. For convenience in this discussion, the K values will be lettered and numbered after the same plan as the extinction values. For instance, K_{PL1} means the constant for a pure ammonium picrate solution when the extinction was read after the first step at 460 mμ, and K_{AH2} means the constant for alpha-TNT at 505 mμ after sulfite hydroxide treatment.

To indicate how the extinction values will change in this analytical procedure, Table II is presented. In this table + indicates a finite extinction value for the constituent, ++ indicate an increase in extinction, ± indicates a decrease, and 0 indicates that a finite extinction is not observable.

A list of equivalents, conversion factors, and K values that have been determined on pure solutions in this laboratory is given below. Although it is believed that the equivalents and conversion factors will hold for other instruments, the K values will vary with the adjustment of the instrument, and so must be determined for ammonium picrate, colored TNT, and alpha-TNT solutions. Although values for these constants are given here, they cannot be used for data on other instruments if the highest accuracy is desired. It will be noted that there is some difference in the constants given here and those given earlier (*5*). This difference is due to a readjustment of the instrument after a slight accident in the laboratory and illustrates the possible variation in constants with different instruments.

EQUATIONS AND FACTORS FOR CALCULATIONS

$$E_{OH1} = E_{CH1} \text{ (as both picrate and alpha-TNT have 0.0 } E \text{ values at 505 m}\mu) \quad (1)$$

$$E_{OL1} = E_{PL1} + E_{CL1} \quad (2)$$

$$E_{OH2} = E_{PH2} + E_{CH2} + E_{AH2} \quad (3)$$

$$E_{CL1} = E_{CH1} \times 1.63 \quad (4)$$

$$E_{CH1} = E_{CH2} \quad (5)$$

$$E_{PH2} = E_{PL1} \times f \text{ or } E_{PL3} \times f \quad (6)$$

where f is a factor depending upon the ammonium picrate concentration and dilution of ammonium picrate:

Under 20 p.p.m., no dilution, $f = 1.92$
20 to 300 p.p.m., $^1/_7$ dilution or higher, $f = 0.3$

$$\text{P.p.m. of colored TNT} = \frac{E_{CL1} - 0.03}{0.170} \times d \quad (7)$$

$$\text{P.p.m. of ammonium picrate} = \frac{E_{PL1} - 0.03}{0.033} \times d \quad (8)$$

$$\text{P.p.m. of alpha-TNT} = \frac{E_{AH2} - 0.03}{0.39} \times d \quad (9)$$

where d in Equations 7, 8, and 9 = the dilution factor.

The simplest way to complete the calculations when all three components are present is on the basis of Equations:

1 and 7 for colored TNT
2, 4, and 8 for ammonium picrate
3, 5, 6, and 9 for alpha-TNT

Although at first glance this spectrophotometric system of analysis may seem slightly formidable, it is really very simple. It is, of course, subject to a number of observational errors, but it presents a sound and practical procedure which enables the estimation of all these constituents when they are present together and indicates the absence of any one that is not present.

BIOCHEMICAL OXIDATION OF AMMONIUM PICRATE WASTE

Concentrations of ammonium picrate in wash waters and evaporation ponds vary between 100 and 300 p.p.m. A series of ammonium picrate dilutions was made up in 1% sewage diluted with mineralized phosphate dilution water and containing concentrations of 0 to 1000 p.p.m. of ammonium picrate. This series was put up in standard B.O.D. bottles and incubated at 20° C. B.O.D. determinations in duplicate were run at the end of 2, 3, and 5 days' incubation, and residual ammonium picrate concentrations were determined by the spectrophotometer for each sample after each incubation period. The results are shown in Table III.

These data indicate: (1) that there is no attack on the above concentrations of ammonium picrate by the microflora of 1% sewage during the 5-day incubation period. (2) As the maximum deviation from the mean B.O.D. for the first five samples at each incubation period was no greater than 10% (which is considered a satisfactory check in B.O.D. work), it is concluded that concentrations of ammonium picrate up to 100 p.p.m. have no effect

n the 5-day B.O.D. of the medium. (3) With higher concentrations of ammonium picrate, an inhibiting effect increasing with he concentration of ammonium picrate is indicated.

To determine the effect of prolonged incubation on the biochemical oxidation of ammonium picrate solutions, 300 p.p.m. of mmonium picrate in 1% sewage were incubated for 50 days and .O.D. determinations were run at regular intervals during the ncubation period. With the concentration of ammonium picrate used, a lower B.O.D. was found at the 5- and 10-day intervals than that of the control, but at the end of the 20-day period here was very little difference. The B.O.D. of the ammonium icrate solutions showed very slight variation from the 15th to he 50th day. The concentration of ammonium picrate as measured by the spectrophotometer was practically unchanged o the 15th day.

EFFECT OF AMMONIUM PICRATE ON ACTIVATED SLUDGE

Fresh filtered sewage containing 300 p.p.m. of ammonium picrate was fed to activated sludge and the usual determinations ere made after intervals of aeration. The results of this experiment (Table IV) show: (1) an immediate loss of 100 p.p.m. f the ammonium picrate dosed, with no further loss during the eriod of aeration. (2) Removal of B.O.D. by activated sludge as retarded after 2 hours' aeration. (3) No significant change n suspended solids was noted.

REDUCTION OF AMMONIUM PICRATE BY SEWAGE

Upon adding ammonium picrate solutions to strong sewages, a eduction of the picrate was observed similar to the reduction of odium hydrosulfite. This reduction is dependent upon the concentrations of ammonium picrate and sewage and the pH of the nedium, the reaction proceeding more rapidly at higher pH alues. The color change is from orange to reddish brown, and, pon long standing, a large reduction in color is noted.

Removal of Ammonium Picrate by Coagulation, Activated Carbon, Soil Percolation, and Sunlight. Tests were arried out using the above methods for removing ammonium icrate from the waste previously described. None of the methds proved economically feasible. Coagulation resulted only in artial removal. Large amounts of activated carbon are required for effective removal. Only one type of soil tested removed an appreciable amount of picrate on the first portion passed through and none removed a finite amount of subsequent portions. Sunlight had no effect on ammonium picrate.

Potability of Drinking Water Containing Ammonium Picrate. Amounts of ammonium picrate as low as 0.5 p.p.m. can be detected in drinking water by taste, 1.0 p.p.m. becomes objectionable, and amounts up to 3.0 p.p.m. render the water unfit for human consumption. These results were arrived at by submitting various concentrations of ammonium picrate in drinking water to twenty persons who were unaware of the contents for appraisal.

Table III. B.O.D. and Ammonium Picrate Concentrations in a 1% Sewage Dilution Containing Increasing Increments of Ammonium Picrate

Sample No.	Ammonium Picrate Concentration, P.P.M.					B.O.D., P.P.M.		
		Residual Determined after				2	3	5
	Dose	Initial	2 days	3 days	5 days	days	days	days
Control	0	0	....	....	...	174	224	278
1	10	8.1	7.5	9.4	9.6	172	223	275
2	20	17.9	16.5	18.9	18.9	182	250	269
3	40	38.8	37.6	40.8	39.6	158	217	278
4	60	58.3	56.8	60.8	58.3	173	223	265
5	100	97.0	94.0	97.9	102.1	182	228	267
		Mean				173	227	272
		Maximum deviation from mean				−15	+23	−7
Control	0	0	....	....	...	128	171	192
6	300	316.0	316.0	300.8	300.8	111	149	162
7	600	635.5	645.5	615.0	625.0	122	129	146
8	800	833.0	865.0	833.0	822.5	118	131	153
9	1000	1083.0	1000.0	1166.0	979.1	108	110	124

Table IV. Effect of Ammonium Picrate on Activated Sludge

	5-Day B.O.D.		Removal		Picrate Remaining	Suspended Solids	
	A	B	A	B	B	A	B
	P.p.m.	*P.p.m.*	*%*	*%*	*P.p.m.*	*P.p.m.*	*P.p.m.*
eed	126.0	152.0	..	..	325.0	..	..
nitial	99.0	101.3	..	..	220.8	..	..
fter 2-hr. aeration	16.7	12.0	83.1	88.1	225.0	..	..
fter 4-hr. aeration	26.9	21.1	72.8	79.1	220.8	..	..
fter 6-hr. aeration	7.0	15.5	92.9	84.7	237.4	2155	2065
fter 24-hr. aeration	2.6	17.9	97.3	82.3	229.2	1930	1865

A = sludge fed with sewage only.
B = sludge fed with sewage plus 300 p.p.m. of ammonium picrate.

Table V. Effect of Time on Reduction of Ammonium Picrate by Domestic Sewage

Days	Total P.P.M. of Reduced and Unreduced Ammonium Picrate
0	22.0
1	10.0
2	13.2
3	10.5
5	10.3
7	10.3
10	9.7
15	9.1
17	7.4
Loss	14.6

SUMMARY

A simple colorimetric or spectrophotometric procedure was found satisfactory for estimating ammonium picrate concentrations in clear wash waters from lines loading ammonium picrate. If the samples are turbid, the samples should be clarified by filtering, settling, and/or centrifuging until free from any turbidity. Chemical flocculation should not be used for clarification unless absolutely necessary, for this may remove some of the picrate.

The qualitative tests for ammonium picrate are reviewed and a technique for a sodium hydrosulfite test which will detect 0.5 p.p.m. of ammonium picrate in the presence of colored and alpha-TNT is presented.

A quantitative three-step spectrophotometric procedure for estimating ammonium picrate, colored TNT, and alpha-TNT in mixed waste waters containing all three is presented. This procedure depends upon extinction readings made at 460 and 505 mμ on the sample after 3 steps: (1) clarification, (2) sulfite hydroxide treatment procedure for TNT wastes, and (3) acidification of the sulfite hydroxide-treated portion. The procedure is simple and direct. After the necessary constants have been determined and the sample is clarified, it enables estimation of all three possible constituents within about 30 minutes. Details of the procedure are given.

A study was made of some of the chemical and biochemical properties of ammonium picrate waste. It was found that the ammonium picrate in the waste has no B.O.D. and that it inhibits the normal B.O.D. reaction of sewage dilutions when present in concentrations over 300 p.p.m. With activated sludge a partial removal of about 30% of the ammonium picrate takes place with the remainder inhibiting the removal of B.O.D. after 2 hours' aeration. Other methods of treatment attempted were of little practical value. Taste tests with a group of people indicated that 1 p.p.m. may be considered the upper permissible limit of ammonium picrate in water for human consumption.

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Determination of Hiding and Contrast-Obliterating Power of Paints

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Determination of the hiding power of paints is commonly based on the application of increasing quantities of paint, until complete (or the same degree of incomplete) concealment of a given design is obtained. Values obtained by different procedures do not agree and in some cases are contradictory: when the quantities applied are increased, the contrast does not increase in the same ratio for different paints. For this reason, every method based exclusively on application of increasing quantities of paint should be rejected.

To determine hiding power, the paint should be applied in normal thickness, establishing the degree of contrast between design and background which may be completely obliterated under these conditions. This may be done by painting on sheets which have a scale of gray shades of increasing darkness, on both a black and a white background, and ascertaining the hiding power by two constants which represent (1) the contrast between the black background and the lightest gray spot which may be concealed completely, and (2) the contrast between the white background and the deepest gray which may be concealed completely, after the paint is applied in normal thickness. To avoid confusion with values obtained by other methods, this type of hiding power may be called contrast-obliterating power.

HIDING power, according to A.S.T.M. D344, is the ability of a paint to reduce the contrast of a black and a white surface to which it is applied (*2*). According to A.S.T.M. D16, it is the power of a paint to render a surface invisible or to cover it up, so that it cannot be seen (*3*). This condition is complied with when designs on the surface are completely concealed.

These two definitions refer to the two more usual methods of determining hiding power. In the first case, paint is applied on sheets which have black geometrical figures sharply outlined on a white background, and the hiding power is indicated by the quantity of paint that just allows the design to be seen against its background (method 1). In the second case, the same test charts may be used. Here hiding power is defined as the least quantity of paint necessary to conceal the designs completely (method 2).

In practice the contrast to be concealed by a paint is generally much weaker, and according to the test, one product may be found inferior to another, yet still good enough to conceal the existing contrasts, so that in practice no difference would be observed. For this reason, the quantity of paint necessary to render a design invisible is often determined on test charts with designs of reduced contrast—for instance, dark gray figures on a light gray background (method 3).

The results obtained with method 2 are often designated as "complete hiding power", and those with methods 1 and 3 as "incomplete hiding power" (*4*, *5*, *6*, *11*, *13*).

The quantities of paint required for complete hiding will be greater than those for incomplete hiding, and in the case of incomplete hiding this quantity will be a function of the degree of contrast that has been covered. But if the values thus obtained are to be employed as a standard for hiding power, for two different paints the ratio of the quantities of paint necessary to give same hiding effect should be constant, independent of the method of comparison.

COMPARATIVE TESTS

For comparative tests, methods 1 and 2 were used, in the expectation that No. 3 would yield results similar to 1.

Paints of different types, colors, and gloss were applied b brush on carton sheets with 8 × 5 white and black square each measuring 25 sq. cm., distributed alternately on a rectangu lar area of 0.1 sq. meter. Several brushouts with increasin quantities of each paint gave a series of sheets from which the ca tons could be selected in which the contrast of the designs ha been reduced to the same degree. These observations were mad with the naked eye after the paint had dried.

In Table I are reproduced the results obtained with three whit enamels of high gloss and three red enamels. The tests whic correspond to the same final contrast are arranged in the sam horizontal lines; lines 1 to 5 and 7 to 10 refer to incomplete, an lines 6 and 11 to complete hiding. In the last two vertical co umns are inserted the ratios of quantities necessary for the sam degree of concealing, which should be constant for each pair paints. But this condition is never fulfilled. For instance, 3 times more of paint *C* is needed than paint *B* for the same degr of incomplete hiding (line 1); whereas the relation is 1.9 f nearly complete hiding (line 5) and 1.5 for complete hiding (lin 6). Comparison of paints *F* and *E* yields a contradictory resul *F* is superior to *E* if the contrast of the surface designs is incor pletely concealed (line 10), and inferior for complete hiding (li 11).

In order to evaluate the significance of these discrepancies, th variation in paint quantities which may be determined for th same hiding effect should be known.

In the case of incomplete hiding power and observation by th naked eye we can estimate this variation by A.S.T.M method D344 (1939) (*2*). This prescribes that the quantities be applied on the test charts shall increase by the factor 1.15 an that 10 intermediate values may be estimated; that means th the uncertainty amounts to $\sqrt[10]{15\%} = 1.3\%$. This precisio

Table I. Quantities of Paint for the Same Hiding Degree

Line No.	Quantities of Paint *A*	*B*	*C*	Hiding	Ratio of Quantities *C* and *A*	*C* and
	Grams/sq. meter					
				White Paints		
1	...	12.1	46	Incomplete, medium contrast	..	3.8
2	...	15.6	55	Incomplete, medium contrast, somewhat less than 1	..	3.5
3	20.7	...	66	Incomplete, medium contrast, somewhat less than 2	3.2	..
4	115	...	166	Incomplete, very small contrast	1.5	..
5	...	95	179	Incomplete, very small contrast, somewhat less than 4	..	1.9
6	180	157	235	Complete	1.3	1.5
				Red Paints		
	D	*E*	*F*		*F* and *D*	*F* an *E*
7	32	...	45	Incomplete, medium contrast	1.4	..
8	37	...	47	Incomplete, medium contrast, somewhat less than 7	1.3	..
9	40	...	50	Incomplete, medium contrast, somewhat less than 8	1.25	..
10	53	68	57	Incomplete, medium contrast, somewhat less than 9	1.1	0.8
11	230	265	280	Complete	1.21	1.1

ble II. Quantities of Paint for the Same Degree of Hiding (6)

Line No.	Quantities of Paint[a] G	H	J	K	Hiding	Ratio of Quantities G and H	G and J	G and K
	Grams/sq. meter							
1	108	26	15	14	Medium, contrast 0.40	4.2	7.1	7.6
2	348	63	60	84	Good, contrast 0.10	5.5	5.8	4.1
3	507	83	129	210	Complete, contrast 0.022	6.1	3.9	2.4

[a] Paints *G*, *H*, *J*, and *K* correspond to products 4, 2, 3, and 1, respectively, Gordon and Gildon.

owever, can be expected only for paints of exactly the same color nd gloss. The least difference in these factors makes much more ifficult the comparison of the test charts. According to tests arried out with a great number of persons of different sexes, ges, and occupations, the uncertainty for red enamels of high loss with very few differences in the red hue, is estimated as at ast 10%.

The difficulty is still greater if we have to determine the quanties necessary for completely concealing the designs. or well-known physiological reasons, the least contrast which ay be perceived with the naked eye corresponds to a smaller eflectance ratio for shades of medium reflectance than for shades f very low or very high reflectance. On the other hand, differnces in color and gloss do not interfere in such a pronounced anner, so that paints of very different color and gloss may be ompared. The author estimates the variation in the deterination of the complete hiding power as at least 15%.

This indicates that the discrepancies deduced from Table I annot be explained by test errors.

These results are confirmed by a paper published by Gordon nd Gildon (*6*), found after the author's experiments had been ompleted. [The author's tests were made in the second quarter f 1944 during the preparation of an Argentine specification for a ethod of determining the hiding power of paints (*Norma ram*, 1072, in preparation). See *Informaciones Iram*, **9**, 159 1945).] Although these authors do not directly treat the prob-em of the present publication, useful values have been extracted rom their tables and curves.

Gordon and Gildon also made brushouts on sheets which had lack and white squares. Using a photometer, they determined he ratio of light reflectance between dark and light squares after pplying increasing quantities of paints of different colors and nishes. (They use the term "contrast ratio" instead of "reflecance ratio", which the author prefers.) In Table II are given he quantities found in their curves.

For medium concealment, leaving a medium contrast ($C = 0.40$; $\Delta D = 0.40$)

For good concealment, leaving a little contrast ($C = 0.80$; $\Delta D = 0.10$)

For almost complete concealment, leaving an almost imperceptible contrast ($C = 0.95$; $\Delta D = 0.022$)

C indicates the reflectance ratio between the dark and the light squares of the painted sheet, and ΔD the corresponding (logarithmic) contrast.

Comparing the quantities of two paints necessary for the same iding effect—that is, the values in the same horizontal line of Table II—the ratios are not constant, nor do they vary always n the same sense, which is in accordance with results deduced rom Table I.

For example, on applying paint *G* in a film 7.6 times heavier han *K*, Gordon and Gildon obtained in both cases the same conrast of $\Delta D = 0.40$ between the dark and the light squares (line 1, Table II); on the other hand, they needed only 2.4 times as nuch of paint *G* as of *K* to render the squares invisible (line 3). The comparison of paint *H* with *J* and *K* yields contradictory results: *H* is superior for covering the designs completely (line 3) and inferior for concealing them only partially (line 1). The producer of paint *H*, consequently, is interested in determining the "complete" hiding power, whereas the manufacturer of *J* and *K* will prefer the "incomplete" hiding power.

The study of hiding power published by the Baltimore Club (*4*) refers only to white paints, so that its results cannot claim such general application as those treated in Tables I and II. Nevertheless Table III gives the values deduced from their curves.

The investigations were carried out by brushouts on test charts provided with black designs on a white background. These show that the relative hiding power of a paint depends on the contrast which is agreed upon for the comparison. The "H. O. lithopone 60% paint" is better than the "lithopone paint" for contrast $\Delta D = 0.10$, but inferior for $\Delta D = 0.022$. In Figures 4 and 6 of (*4*), the discrepancies pointed out occur even with paints which contain the same pigment and the same vehicle, the only variation being the ratio between these components. According to Figure 6 the "H. H. lithopone paint" with 50% pigment concentration covers better than that with 40% if a reflectance ratio of less than 0.96 is to be obtained (ΔD greater than 0.018, "incomplete hiding"), whereas for a ratio greater than 0.96 ("nearly complete hiding") the 40% paint is superior.

Another confirmation of the author's results may be found in Haslam's paper (*7*). He indicates that the same quantities of two different white paints, I and II, are necessary to cover up a black background completely; but to conceal it incompletely, leaving the same light reflectance, with both paints, he had to apply a greater quantity of II than of I, the ratio depending on the degree of obliteration. For this reason, Haslam rejects the determination of incomplete hiding power.

According to all the above experiments, it is not permissible to stipulate a simple proportion for transforming incomplete hiding power into complete hiding power, as is assumed by the formula (*5*, p. 25).

$$\frac{\text{Incomplete H. P. of sample}}{\text{incomplete H. P. of standard}} = \frac{\text{complete H. P. of sample}}{\text{complete H. P. of standard}}$$

All the arguments adduced prove that the hiding power values for paints depend on the degree to which the original contrast of the designs on the test chart has been reduced. There is no general value for the hiding power, neither absolute nor relative, if this magnitude is defined and determined by the methods to which reference has been made and which are the most commonly used.

Table III. Quantities of Paint for the Same Degree of Hiding (4)

Paint	Quantities of Paint $C = 0.80$, $\Delta D = 0.10$	$C = 0.95$, $\Delta D = 0.022$	Figure of (*4*)
	Grams/sq. meter		
Titanium-lithopone, 50%	3.2	8.9	2
Titanium-barium, 50%	3.9	11.2	4
H. O. lithopone, 60%	4.1	12.3	3
Lithopone	4.4	10.4	1
L. O. lithopone, 60%	5.6	13.2	5
H. H. lithopone, 40%	5.7	12.8	6

CAUSES OF DISCREPANCIES

The causes of the discrepancies pointed out above are the same as those mentioned to explain the effect of pigment concentration on hiding power (*4*). The concealing effect of a pigment depends not only on its quantity per unit area, but also on its dispersion and distribution within the layer; and the concealing behavior of a paint coat is determined by its light absorption as well as by light scattering. If we apply a greater quantity of paint, we may expect the areal pigment concentration and its light absorption to increase in the same ratio, but the scattering will change for

each paint in a different manner, thereby causing the discrepancies mentioned above.

The author is indebted to Deane B. Judd, National Bureau of Standards, for the suggestion that Kubelka and Munk's study (*10*) of the relationship between light reflection and thickness of homogeneous layers might aid in this investigation. The latter expounded an equation expressing this relationship by two constants: reflectivity—the reflectance of an infinitely thick specimen of the material—and coefficient of scatter. Judd, Hickson, and Eickhoff (*9*) showed that this equation applies sufficiently well to white and near-white cold-water paints. If it could be proved that the theory of Kubelka and Munk is also valid for oil paints, both glossy and flat, it would be possible to base the specification of hiding power of paint simply on the scattering coefficient, and the discrepancies pointed out above would not exist. This decision would have to be checked by brushing out known quantities of paint over black and white backings, and measuring the reflectances.

Judd (*9*) gives a curve chart calculated by means of the Kubelka-Munk equation, which permits in a very simple manner deduction of the reflectivity and the product of thickness and scattering coefficient, from the ratio of the reflectances on black and white backgrounds and from the reflectance on a black background; knowing the thickness we may calculate the scattering coefficient. Once these two constants—reflectivity and scattering coefficient—are determined we may find with the aid of the same curve or the corresponding equations, the values of light reflectance on any background (black, white, or gray) and for every desired thickness of film. These two numbers would, consequently, characterize the hiding behavior of the paint in a general manner.

In practice, however, this method would have two disadvantages. Apart from the necessity for using a reflectometer, results obtained by measuring the reflectances with daylight would be satisfactory only in the case of white or near-white paints; materials having highly chromatic colors will require values of reflectivity and coefficient of scatter as functions of wave length (*9*).

SUGGESTED NEW METHOD

General Principles. The determination of hiding power is usually carried out by applying increasing quantities of paint, until complete—or the same degree of incomplete—concealment of a design is obtained. In order to secure the desired covering, the paint must sometimes be applied in a thickness which is far from normal; values determined under such extreme conditions are not of practical interest, as they give no indication of the hiding behavior of the paints under normal conditions of painting.

In view of this fact, it seems best to face the problem from the inverse aspect. We should not raise the usual question "what thickness of paint is to be applied for concealing a given contrast (partially or completely)?", but should ask "which contrast may be concealed when the paint is applied in normal thickness?".

The A.S.T.M. method (*2*) is based on this second point of view. But this test has to be carried out in a rather inconvenient manner and for this reason has not received wider acceptance (*6*). According to this method, paint should be applied in a normal thickness on a white sheet with black designs, and the concealing degree determined by visual comparison with a scale of contrasts obtained by brushouts of increasing quantities of a standard paint on other sheets which have the same designs. This method is not an absolute one and requires a standard paint; satisfactory results can be obtained only with products of very similar hue and finish.

In order to avoid all these inconveniences, a new method for the determination of hiding power is proposed here. The paint is to be applied in normal thickness, establishing the degree of contrast between background and design which may be completely obliterated under these conditions. In order to comply with this stipulation, paint is applied on test charts which have, on both a black and a white background, a scale of gray shades of increasing darkness and the hiding power is ascertained by two constants which are numerically equal to the value of (1) the contrast between the black background and the lightest gray spot which may be concealed completely, and (2) the contrast between the white background and the darkest gray spot which may be concealed completely, when the paint is applied in a normal degree of thickness. As a consequence of these definitions, we obtain positive values for the standards if the test is carried out on a black background, and negative ones for tests on a white background.

This contrast is found as the difference of the densities of background and gray spot, the density being the negative logarithm of the light reflectance ratio of these two areas. In consequence the "contrast" may also be defined as the negative logarithm of the ratio of the light reflections of background and gray spot. Many papers indicate directly the numerical value of this reflectance ratio (sometimes called contrast ratio, although the term "contrast" itself involves a ratio) in order to describe the contrast between two areas, but according to the law of Weber and Fechner the impression on the human eye corresponds to the densities and their difference, and not to the reflectances and their ratio.

Although the term "contrast" should be reserved for the density difference, the term "logarithmic contrast" is sometimes found, since this difference is equal to the logarithm of the reflectance ratio and in order to differentiate from the "numerical contrast", which is the numerical value of the reflection ratio.

The magnitudes mentioned are defined and connected to one another by the following relations:

If I is the intensity of the incident light, and I', I'', etc., are intensities of the reflected light, we have:

Light reflectance: $R' = \frac{I'}{I}$

Density: $D' = \log \frac{I}{I'} = -\log R'$

Reflectance ratio (contrast ratio, numerical contrast): $C = \frac{I'}{I''}$

Contrast (logarithmic contrast): $\Delta D = D' - D'' = \log \frac{I''}{I'} = -\log$

In scientific photography the magnitudes "density" and (logarithmic) "contrast" are used exclusively, never the "reflectance" and their ratio (the numerical contrast).

Whenever we wish to differentiate the reflectances, densities, reflectance ratios, and contrasts which exist in the surface to be painted, from those which exist in the applied paint coat, we use

R, R', D, D', C, ΔD, etc., for magnitudes which refer to the background

and

$R_{R'}$, $D_{D'}$, $C_{R'}$, $D_{D'}$, etc., for magnitudes which refer to the paint film

indicating, for example, $D_{D'}$ (or $D_{0.50}$), the density of a paint film which has been applied on a surface, the density of which is D (or 0.50).

To avoid confusion this type of hiding power might be called "contrast-obliterating power" since usually "hiding power" is understood to be the quantity of paint necessary for a definite hiding effect, whereas according to the present method a contrast value is used for measuring the concealing behavior.

If it is more convenient, the gray scale may also be on a uniform gray background. In this case, the hiding behavior of the paint would be characterized by two constants which are numerically equal to the contrasts existing between the gray background and the lightest and darkest gray spots which have been rendered invisible, the first value being a positive number and the second a negative one. For reasons explained later

this paper is restricted to measurements with black and white backgrounds.

The new method gives absolute, not relative values (as, for example, the A.S.T.M. method and many others); no standard paint is necessary. It is independent of the color and the finish of the paint. It may be carried out in a simple manner, with a single application of paint, whereas for the other proceedings it is generally necessary to paint a greater number of test charts in order to obtain one with the required reduction of the original contrast. The observation is carried out by the naked eye, without any optical instrument, on both fresh and dried films.

Thus the values of contrast-obliterating power are not only more closely related to the practical performance of the paint than the conventional hiding power, but the determination may be carried out more easily.

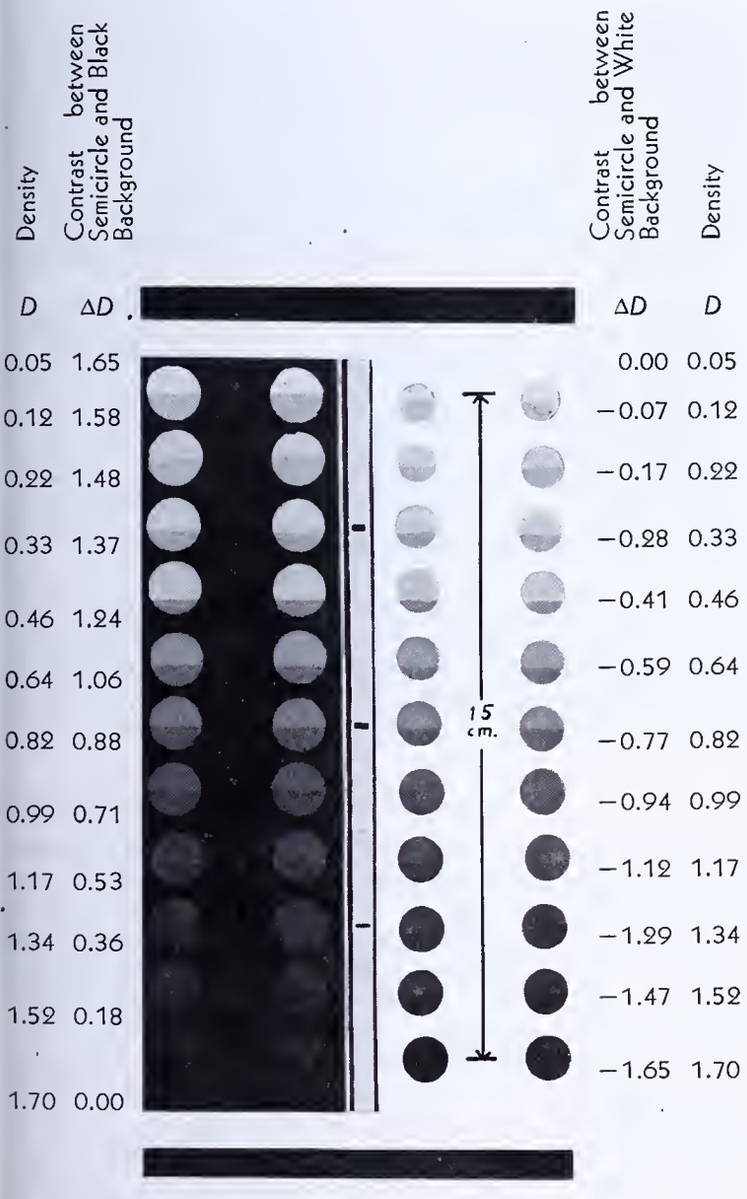

Figure 1. Test Chart for Determining Contrast-Obliterating Power

THE METHOD. Figure 1 shows a gray scale prepared by photography, in which the gray shades have the form of semicircles in order to make observation easier. The first semicircle is white, whereas the remaining part of the circle is a very light gray. The same gray is repeated in the upper semicircle of the next sphere, the adjoining semicircle following with a little deeper gray.

In a darkroom, a fine-grained film of soft graduation was covered with a carton perforated in a manner which corresponds to the circles of Figure 1. A weak light was mounted at such a distance that with a 4-second exposure and after normal development, very little blackening was brought about. With this illumination, the rows of circles were exposed, the exposure time being augmented so that each row was blackened very little more than the preceding (time factor for film was $\sqrt[3]{2}$). A contact copy was prepared from the negative, utilizing a paper of very hard graduation and a special developer in order to obtain blacks of a high density. Adapting the exposures and developments to the special characteristics of the photographic material, columns of gray spots were obtained, varying from a very light to a very dark gray. In order to have the circles on a white background and to obtain a white semicircle, the corresponding part of the negative was covered with the special photographic opaque color commonly used for this purpose.

If many test charts are to be prepared it is convenient to have them made by lithography. Each copy of the set has the same densities of the white, gray, and black spots, and of the backgrounds, so that the density measurements of one test chart are good for all.

In this manner, the gray of the semicircles gradually increases in darkness, the last one being black. The gray of the lower semicircle is always repeated in the upper part of the next circle in order to facilitate control of the reading. This arrangement has been repeated in four columns, two on a white background, and two on a black. The four semicircles in the same horizontal line have the same gray, but by a contrast effect those on the white backing seem darker. The vertical black lines and the two horizontal black blocks have been included so as to make possible controlling the uniformity of the paint film.

On the two frames are indicated the densities, D, of the different grays, determined by the Goldberg densitometer. The contrasts, ΔD, which exist between the black (left) and the white (right) background and each gray spot, are also given.

In order to obtain satisfactory results with this method, it is necessary to apply the paint in a layer of exactly uniform thickness. Such films have been produced by the use of the Bird film applicator, otherwise known as the Bradley blade. [In a general description of methods of applying uniform films (*12*), this doctor blade is dealt with; a sketch of this applicator may be seen in Figure 591 of Gardner's publication (*5*). A special method was described by Howard (*8*). See also (*1*).]

This device is an accurately machined steel bar with a bevel edge and a specific clearance between the edge and the ends. For these tests three different applicators were used, one with a clearance of 0.0075 ± 0.001 mm. and a width of 5 cm., another with a clearance of 0.150 ± 0.002 mm. and a width of 5 cm., and a third with a clearance of 0.150 ± 0.002 mm. and a width of 15 cm.

The thickness of the films obtained with these blades depends to a certain extent on the characteristics of the paint—for example, viscosity, flowing—and the manner of operating. But the differences of film thickness are not so pronounced as in the case of brush application.

Painting enamels A and D normally with a brush, fresh films 0.07 and 0.04 mm. thick, respectively, were obtained. With brushouts well spread out, paint A also gave a thickness of 0.04 mm. On the other hand, the applicator rendered the same thickness with both paints, 0.042 mm. with the clearance of 0.075 mm. and 0.074 mm. with the clearance of 0.150 mm. It may therefore be assumed that the films obtained with the two clearances of 0.075 and 0.150 mm. correspond for these paints to a well brushed out layer and to a heavy coat, respectively; which means in both cases the thickness of one coat in the normal paint job.

In order to have the photographic papers well flattened—necessary for uniform application with the Bradley blade—it was fixed on a glass plate with a nondrying adhesive which could be easily washed off once the test had been finished. Howard (*8*) recommends holding the test charts flat by suction on a plane plate-glass cover drilled with holes.

PREPARATION. Heat 34 parts of glycerol to the boiling point, add 51 parts of white dextrin, mixing well, and, after cooling, 14

parts of water and 1 part of turkey red oil or the corresponding quantity of another wetting agent.

PRACTICAL RESULTS. Figure 2 shows the test chart after a paint has been applied in the manner described, and in Table IV are reproduced some results of tests. A higher absolute value indicates that the product has a superior hiding power, the positive values corresponding to the obliteration of spots lighter than the background, and the negative ones to spots darker than the background.

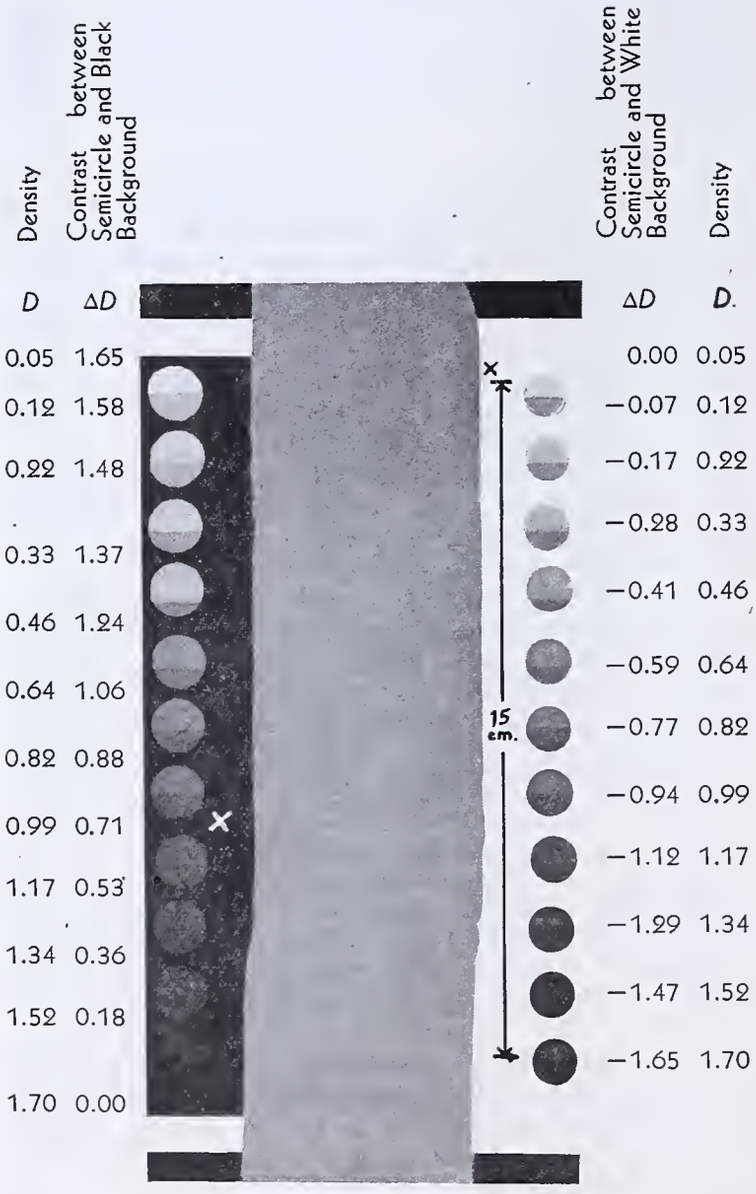

Figure 2. Test Chart after Applying Paint

Semicircles with the greatest contrast which have been completely obliterated are marked with a cross; they correspond to a contrast $\Delta D = 1.26$ on the black background, and greater than -0.07 but less than 0 on the white background. If the paint is applied with a blade of 0.075-mm. clearance, the tested product is characterized by

$$COP_{C=0.075} \text{ on black} = 1.26$$
$$COP_{C=0.075} \text{ on white} > -0.07 \quad < 0.00$$

As the paints tested showed in practice considerable differences in hiding behavior, it may cause surprise that according to Table IV no differences were found when the products were well sprayed out on a gray scale appearing on a white background. In this case, the obliterated contrast is still smaller than that of the lightest gray spot. If any differences exist, they must be within the very small contrast range of $\Delta D = 0$ and $\Delta D = -0.05$, and therefore not determinable by the test chart. Applying the same paint in a thick coat, the obliterated contrast is great enough to

Table IV. Contrast-Obliterating Power of Different Paints

(Greatest contrast obliterated by a paint film of normal thickness)

Paint	Color	Greatest Contrast Obliterated: Well Brushed Out[a] White background	Well Brushed Out[a] Black background	Thick Coat[b] White background	Thick Coat[b] Black background
D	Red	−0.05	0.78	−0.07	1.05
E	Red	....	..	−0.05	0.58
F	Red	−0.05	0.39	−0.05	0.84
L	White	−0.05	0.72	....	..
M	White	....	..	−0.05	1.01
N	White	−0.05	1.01	−0.09	1.15
P	Yellow	−0.05	0.72	−0.07	1.15
Q	Bordeaux-maroon	−0.05	0.36	−0.59	0.88

[a] Applicator clearance, 0.075 mm.
[b] Applicator clearance, 0.150 mm.

be stated in this chart, but the values for the different products are very similar and give no true picture of their real hiding differences. On the other hand, very noticeable differences exist for the contrast values which may be obliterated on the black background, and these results agree with the differences of hiding behavior which were observed in the practice of painting for the tested products. Consequently, the determination of contrast-obliterating power should be carried out on a black background.

Table IV presents another proof that the concealing power of a paint does not increase in the same degree for the different products when applied with greater thickness. On the black background, paints *N* and *P* cover in the same degree for a "thick coat", but for a "well brushed out" layer *P* is very inferior to *N*.

An exceptional effect occurs with paint *Q*. For the lesser thickness, *Q* has the least contrast-obliterating power of all products on both backgrounds; for the greater thickness, *Q* is very superior to all the other paints of Table IV on the white background, but not on the black. This example proves the importance of testing also on a white background.

For this reason, it is preferable to indicate two values of contrast-obliterating power, one referring to a black background, and the other to a white one (Figure 2), but in general, characterizing a paint by its contrast-obliterating power on a black background may be sufficient.

GENERAL REMARKS

Gardner (*5*) gives on page 19 a hint on the method suggested here—i.e., to determine the degree of contrast which may be completely obliterated. This proposal was apparently never put in practice, for Gardner would have observed that the test in the form suggested by him produces rather unsatisfactory results. Gardner proposes covering with the test paint a series of gray blocks of increasing density, which alternate with white blocks; which means a test chart in which different gray shades appear on a white background. In this way, as may be seen from Table IV, only very pronounced differences of hiding power can be observed. It is imperative to observe the concealment of contrasts between the single gray degrees and a black background.

Others may prefer a gray background, several gray backgrounds of different shades, or black and white and grays, arguing that one or the other of these proposals more nearly approaches practical conditions. In this case, however, the gray shades would have to be well defined, and the advantage would possibly be annulled by difficulty of obtaining the shade agreed upon wherever the test should be carried out.

Considering the fact that the author's method is designed for practical purposes, we must limit to the minimum the number of constants used to characterize the hiding behavior and choose among the different possibilities those which may be put in practice in the easiest manner, because a somewhat less exact characterization is satisfactory if we may obtain a sufficiently good one

with fewer samples and less work. Finally, it is shown below that for theoretical reasons also the use of gray backgrounds is inadmissible.

In the test chart the author has utilized ten gray shades as well as black and white. It is not necessary to use the same graduation as in this test chart. A finer graduation is possible, but should not be necessary for practical purposes. If required, intermediate values may be obtained by interpolation.

Preparing test charts in different places, it is certain that other gray shades will be obtained than those which appear in the author's chart. But these changes are without importance because the contrast-obliterating power values refer to the contrast values which have been determined for the gray spots, and not to the number of spots obliterated. These contrasts are defined as differences of densities; the measurement need be carried out only once for every set of test charts and may be done in any physical or scientific-photographic laboratory.

The author preferred in his test chart gray spots which are surrounded by black or white in order to make the observation of obliterating easier. Only a limited number of contrasts appears in the test chart (which according to the author's opinion is sufficient), but it would likewise be possible to use a gray stripe, the depth of which increases continuously. Such a design can be prepared, for example, by exposing a photographic material behind a rotating opaque disk from which a sector has been cut out, the width of which increased from the center to the periphery by a factor which depends on the characteristics of the photographic material.

The method is to be carried out with normal thickness of paint film. This normal thickness is the most convenient for application, but not necessarily equal for all products. It might therefore be better to use a series of applicators with corresponding clearances (or one with variable clearance), but the author prefers to test paints of similar type with the same thickness—such as is obtained by the use of an applicator—because the painter generally levels the differences of the commercial products by dilution and his manner of working.

To avoid misunderstandings respecting thickness, it is advisable to indicate the clearance of the applicator used, and if the paint has been diluted for application to describe in what manner this has been done.

For example, according to Table IV, paint D should be characterized by

$$COP_{c\,=\,0.075} \text{ on black} = 0.78$$

which means that by paint D, applied with a blade of the clearance of 0.075 mm., a gray spot may be obliterated the contrast of which is $\Delta D = 0.78$.

With the knowledge that the paint film in this case has a thickness of 0.042 mm., the same paint may be described by

$$COP_{t\,=\,0.042} \text{ on black} = 0.78$$

Values obtained by the usual methods may lead to conclusions concerning the quantities of paint which give the same concealing result, whereas the suggested method permits no correlation with paint quantities. But the experiments described at the beginning of this paper show that such an advantage does not exist, at least for paints of greater hiding differences (and in the case of products of little hiding differences, the method may yield the same correlation). Results obtained with a certain film thickness hold only for that particular thickness, and therefore varying results may be obtained from tests where the film thickness is very different. Quantitative statements are of interest for practical purposes only if they refer to entire coats of a normal thickness, because the painter does not apply the paint in a thickness which is a fraction of one coat, although he may to a certain degree change the paint quantity by dilution and manner of working. The hiding behavior of films of the thickness of a normal coat is the problem to be solved by the suggested method.

If paints applied in normal thickness conceal the greatest contrast which exists in the author's test chart, they must be considered as best, and it will be of no practical interest if differences arise in hiding behavior when these products are applied in a thinner coating.

The tests are not necessarily to be carried out with the same thickness for every kind of paint, but the thickness should correspond to normal conditions of painting. This thickness, however, may change within certain limits. For this reason, in the case of paints of similar hiding power, increasing quantities of paint may be used, in order to obtain values which allow conclusions concerning the quantities which give the same concealing result, but only the relatively small variation of thickness is permitted which corresponds to those occurring in the practical paint job for one coat, as a consequence of different manners of diluting and working.

The same result will not always be obtained from two coats of thickness t as from one coat of thickness $2 \times t$. Nor is the relation of hiding behavior of two paints the same for one coat as for two coats. For this reason, it will sometimes be necessary to determine the contrast-obliterating power for one and for two coats. In this case the first coat is applied with the applicator 15 cm. wide, and the second one, after the first one is dry, with the 5-cm. applicator.

For example,

$$COP_{o\,-\,2\,\times\,0.150} \text{ on black} = 1.59$$

means that the tested paint, applied with a doctor blade of 0.150-mm. clearance and in two coats, will obliterate a gray shade on a black background which has a contrast of 1.59.

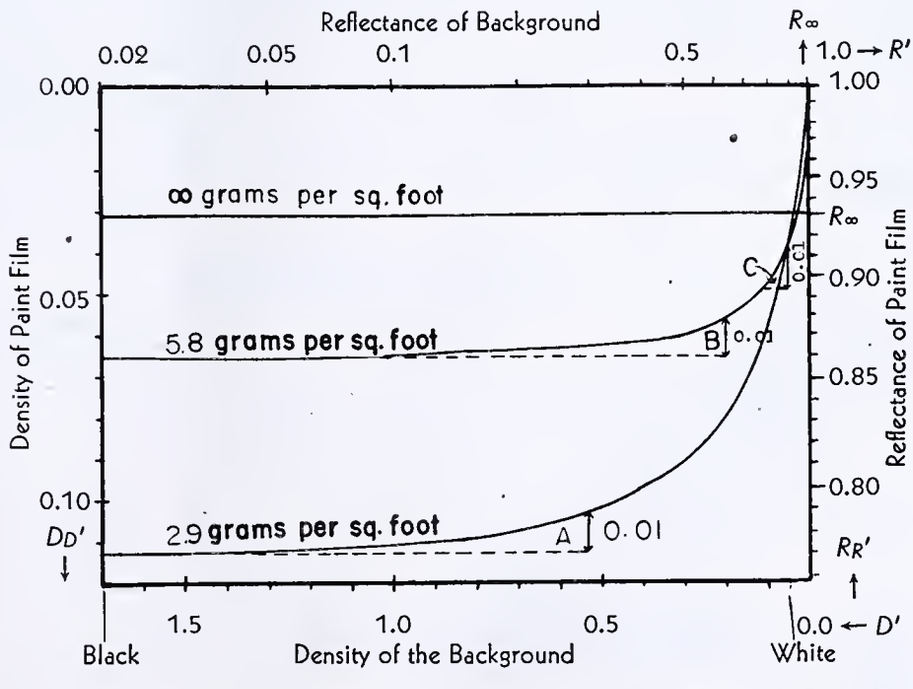

Figure 3. Density $D_{D'}$ (Reflectance $R_{R'}$) of Paint a_1 Applied in a Coat of Constant Thickness on Background of Density D' (Reflectance R')

Calculated according to Kubelka-Munk theory with constants found by Hickson and Eickhoff. Gray areas correspond to threshold zones (*A*, light gray; *B*, gray; *C*, dark gray).

Table V. Light Reflectance of White Cold-Water Paints

(According to Hickson and Eickhoff)

Paint	Thickness G./sq.ft.	Light Reflectance of Paint on Black Background R_0	Ratio of Reflectances on Black and White Backgrounds $C_{0.80}$	Reflectance of Infinitely Thick Paint Film R_∞	Average Value R_∞
a_1	2.9[a]	0.770	0.880	0.940	0.931
a_1	5.8[b]	0.860	0.960	0.922	
c	2.9[a]	0.780	0.920	0.865	0.870
c	5.8[b]	0.850	0.980	0.875	

[a] 31 grams per sq. meter.
[b] 62 grams per sq. meter.

INTERPRETATION OF CONTRAST-OBLITERATING POWER BY THE KUBELKA-MUNK THEORY

Judd, Hickson, and Eickhoff (*9*) have proved that the hiding behavior of white or near-white cold-water paints may be described by the Kubelka-Munk equation (*10*). It should therefore be possible to expound by this theory the significance of the standard proposed for that characteristic of paints, at least for the paint type mentioned. For this study the author took advantage of Judd's equations and the measurements by Hickson and Eickhoff (*9*).

According to Judd's Equations 2a and 2b, the reflectance ratio, $C_{R'}$, may be expressed by the following equation:

$$C_{R'} = \frac{R_0}{R_{R'}} = \frac{1 - R'R_0}{(1 - R') + \dfrac{R'(1 - R''R_0 - C_{R''} + R''C_{R''})}{R''C_{R''}}} \quad (1)$$

when

R' and R'' are light reflectance of the background
$R_{R'}$ and $R_{R''}$ are light reflectance of a paint film applied on a background, reflectance of which is R' and R'', respectively
R_0 is light reflectance of a paint film applied on a background, the reflectance of which is 0 (black background)

$$C_{R'} = \frac{R_0}{R_{R'}} \text{ and } C_{R''} = \frac{R_0}{R_{R''}}$$

are reflectance ratio (contrast ratio)

Equation 1 may be written also as:

$$R_{R'} = \left(1 + \frac{(1 - R''R_0 - CR'')\,R'}{R''C_{R''}}\right) \times \frac{R_0}{1 - R'R_0} \quad (2)$$

The Hickson-Eickhoff results refer to paint applied on a black and white background. In the latter case, the reflectance being $R'' = 0.80$, we obtain for this special case

$$R_{R'} = \left(1 + \frac{(1 - 0.80R_0 - C_{0.80})\,R'}{0.80\,C_{0.80}}\right) \times \frac{R_0}{1 - R'R_0} \quad (3)$$

From the curves plotted from the Hickson-Eickhoff Tables V and VI, for the values for R_0 and $C_{0.80}$ reproduced in Table V were obtained. Indicated there is also the "light reflectivity" R_∞—that is, the light reflectance of an infinitely thick paint film—this magnitude has been determined from Figure 2 of Judd's paper.

Substituting in Equation 3 the magnitudes R_0 and $C_{0.80}$ from the figures of Table V, we obtain the following formulas:

For paint a_1 and thickness 2.9 grams per square foot

$$R_{R'} = \frac{(1 - 0.704R') \times 0.770}{1 - 0.770R'}$$

For paint a_1 and thickness 5.8 grams per square foot

$$R_{R'} = \frac{(1 - 0.843R') \times 0.860}{1 - 0.860R'}$$

For paint c and thickness 2.9 grams per square foot

$$R_{R'} = \frac{(1 - 0.712R') \times 0.780}{1 - 0.780R'}$$

For paint c and thickness 5.8 grams per square foot

$$R_{R'} = \frac{(1 - 0.842R') \times 0.850}{1 - 0.850R'}$$

These four expressions enable us to calculate the light reflectance, $R_{R'}$ of a paint film of thickness 2.9 and 5.8 grams per square foot, respectively, which has been applied on a background the reflectance of which is R'. The results have been used to plot the curves of Figures 3 and 4. Since these curves are intended to expound an optical-physiological phenomenon, they are plotted to a logarithmic scale, according to the law of Weber and Fechner. The transformation of the (numerical) reflectances, R' of the background and $R_{R'}$ of the paint films, to the (logarithmic) densities, D', of the background and $D_{D'}$ of the paint film has to be made according to the relation:

$$\text{Density} = -\log \text{reflectance}$$

The curves of Figures 3 and 4 represent the density, $D_{D'}$ (reflectance $R_{R'}$) of a paint film of constant thickness which has been applied on a background of density D' (reflectance R').

Figure 3 shows that paint a_1, applied in a constant thickness of 2.9 grams per square foot, gives a density of 0.113 on a black background. On backgrounds of less depth, gray backgrounds, this density decreases more and more as the background turns lighter, being $D_{D'=0} = 0.004$ on an ideal white background. The difference of light reflection, however, cannot be noticed until the density has become modified to a certain degree, which

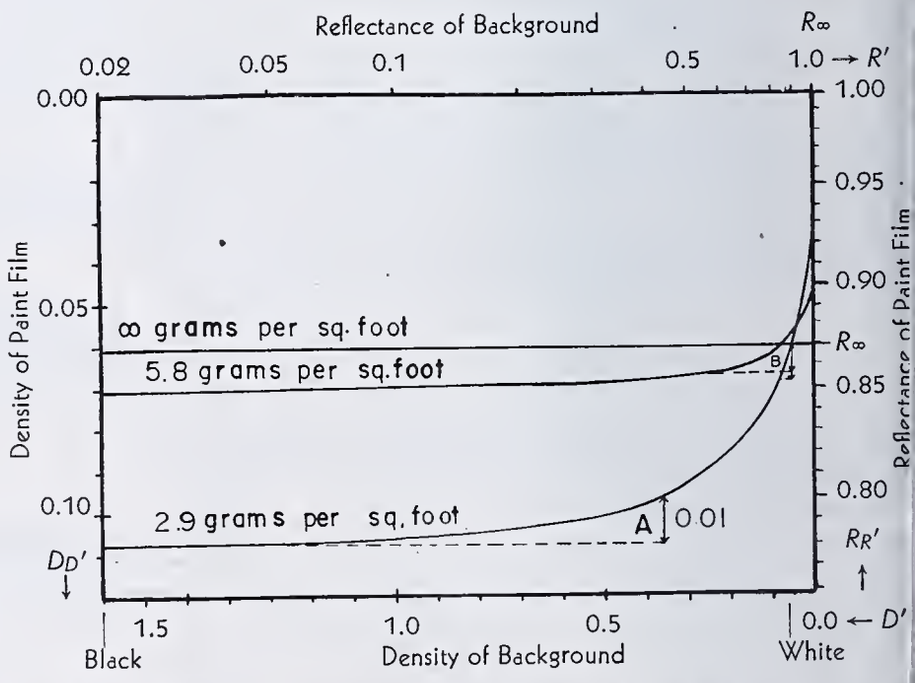

Figure 4. Density $D_{D'}$ (Reflectance $R_{R'}$) of Paint c Applied in a Coat of Constant Thickness on a Background of Density D' (Reflectance R')

Calculated according to Kubelka-Munk theory with constants found by Hickson and Eickhoff. Shaded areas (*A*, light gray; *B*, dark gray) correspond to threshold zones.

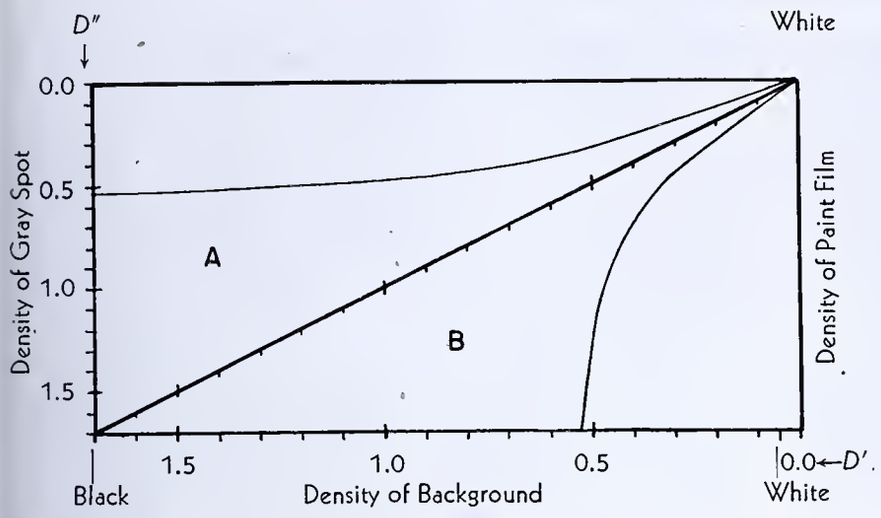

Figure 5. Densities D'' of Gray Spots Obliterated by Paint a' Applied in a Coat of 2.9 Grams per Square Foot on a Background of Density D'

Calculated from Figure 3. Gray areas correspond to obliteration of gray spots lighter (*A*, light gray area) and darker (*B*, dark gray area) than background.

is commonly called "threshold value". The author estimates the threshold contrast for this case at approximately $\Delta D_{D'} = 0.01$. Since the paint film density on the black background is $D_{1.70} = 0.113$, we observe, the first reflectance difference with a film density of $D_{D'} = 0.113 - 0.01 = 0.103$. Figure 3 shows that such a reflectance occurs if the paint has been applied on a background that has a density $D' = 0.53$. Therefore, the deepest gray spot that may just be obliterated on a black background of density $D' = 1.70$, has density $D' = 0.53$, which means

$$\text{COP}_{t\,=\,2.9 \text{ grams per sq. foot on black}_{D'\,=\,1.70}} = 1.70 - 0.53 = 1.17$$

In order to facilitate these readings, in Figures 3 and 4 the threshold zones, which correspond to the black background ($D' = 1.70$; $R' = 0.02$) and to the white background ($D' = 0.05$; $R' = 0.89$), are indicated.

In the manner described, the following contrast-obliterating power values were obtained:

For paint a_1 (Figure 3)

$\text{COP}_{t\,=\,2.9}$ grams per square foot

On black$_{D'\,=\,1.70}$	$1.70 - 0.53 = 1.17$
On white$_{D'\,=\,0.05}$	$0.05 - 0.07 = -0.02$

$\text{COP}_{t\,=\,5.8}$ grams per square foot

On black$_{D'\,=\,1.70}$	$1.70 - 0.20 = 1.50$
On white$_{D'\,=\,0.05}$	$0.05 - 0.11 = -0.06$

For paint c (Figure 4)

$\text{COP}_{t\,=\,2.9}$ grams per square foot

On black$_{D'\,=\,1.70}$	$1.70 - 0.36 = 1.34$
On white$_{D'\,=\,0.05}$	$0.05 - 0.08 = -0.03$

$\text{COP}_{t\,=\,5.8}$ grams per square foot

On black$_{D'\,=\,1.70}$	$1.70 - 0.07 = 1.63$
On white$_{D'\,=\,0.05}$	$0.05 - 0.25 = -0.20$

According to these results, paint c hides better than a_1, the difference being not very pronounced. This agrees with the result computed by Hickson and Eickhoff on the basis of amount required for incomplete hiding (method 1). However, such agreement will not always occur.

In order to complete Figures 3 and 4, the curves for an infinitely thick layer of paint which has reflectance R_∞ (Table V) were also plotted; the reflectance of the paint film in this case being independent of that of the background, straight lines parallel to the abscissa are obtained.

If the paint is applied on a background, the reflectance, R', of which is just equal to R_∞, the resulting reflectance, $R_{R'}$, of the coat cannot be other than the original one of the background, independent of the film thickness; this means that all the curves plotted for different thicknesses must pass through a point, the coordinates of which are $R' = R_\infty$, $R_{R'} = R_\infty$, a condition which is sufficiently well complied with in Figures 3 and 4.

With the aid of these curves, we may also calculate contrast-obliterating power values for every gray background, as is shown by the following example:

We observe in Figure 3 that a paint film of the constant thickness 2.9 grams per square foot gives a density $D_{D'} = 0.097$, if applied on a gray background, the density of which is $D' = 0.40$. The threshold value being $\Delta D_{D'} = 0.01$ we may, in consequence, expect to distinguish from this paint film only coats which have densities, $D_{D'}$ greater than $0.097 + 0.01 = 0.107$ and less than $0.097 - 0.01 = 0.087$. The curves indicate that paint films of densities $D = 0.107$ and 0.087 are obtained on gray backgrounds of densities $D' = 0.70$ and $D' = 0.26$, respectively. That means: if we apply paint a_1 in a thickness of 2.9 grams per square foot on a gray background that has a density of $D' = 0.40$, we may obliterate all spots darker than the background, which have densities between $D' = 0.40$ and $D' = 0.70$, the corresponding densities for spots lighter than the background being between $D' = 0.40$ and $D' = 0.26$. Therefore, we obtain the following values for the contrast-obliterating power:

$$\text{COP}_{t\,=\,2.9 \text{ grams per square foot on gray}_{D'\,=\,0.40}} \begin{cases} 0.40 - 0.26 = 0.14 \\ 0.40 - 0.70 = -0.30 \end{cases}$$

Of these two values, as usual, the positive refers to the obliteration of spots lighter than the background, and the negative to spots darker than the background.

In the same manner, the densities, D'', of the lightest and the darkest gray spots which may be obliterated on backgrounds of different gray shades were calculated. Figure 5 shows the results for the Hickson-Eickhoff paint, a_1, applied in a thickness of 2.9 grams per square foot. The areas which correspond to obliteration are shaded. On a gray background of $D' = 0.50$ all spots which have densities between $D'' = 0.32$ and $D'' = 1.24$ are obliterated, resulting in:

$$\text{COP}_{t\,=\,2.9 \text{ grams per square foot on gray}_{D'\,=\,0.05}} \begin{cases} 0.50 - 0.32 = 0.18 \\ 0.50 - 1.24 = -0.74 \end{cases}$$

On a gray background of density $D' = 0.525$, the obliterated gray spots may have a density between $D'' = 0.325$ and $D'' = 1.70$—i.e., black—which means

$$\text{COP}_{t\,=\,2.9 \text{ grams per square foot on gray}_{D'\,=\,0.525}} \begin{cases} 0.525 - 0.325 = 0.20 \\ 0.525 - 1.70 = -1.175 \end{cases}$$

With this background of density $D' = 0.525$, we have for our special case reached the limit for the obliteration of spots darker than the background. For all backgrounds with a deeper gray density, we have always the same density value of $D'' = 1.70$ (black) for the darkest obliterated spot. Carrying out the test in the areas beyond this limit-density, we would consequently find for all paints—independent of their special characteristics and the film thickness—contrast-obliterating power value which is equal to the difference of the density value of the background and 1.70; for the background with the density $D' = 0.80$, for example, we would obtain in all cases $\text{COP} = 0.80 - 1.70 = -0.90$.

The contrast-obliterating power values obtained in the manner described are shown graphically in Figure 6. These curves, although describing the special case of white cold-water paints, give a true and very general picture of the significance of the contrast-obliterating power. The values are in all cases very small if a white background is used: the same result obtained with experiments described above. On the other hand, the calculated curves show—also in agreement with the practical tests—that the contrast-obliterating power values vary in a pronounced manner if the contrasts refer to a black background.

The dot-dash curve of Figure 6 refers to the limit case, indicating that the tests always give the same contrast-obliterating power values for spots darker than the background, for every paint and film thickness, once the limit depth of the gray background has been passed. In the special case, these limits are according to Figure 6:

For paint a_1 and thickness 2.9 grams per square foot

$$D' = 0.53 \ (R' = 0.30)$$

For paint a_1 and thickness 5.8 grams per square foot

$$D' = 0.20 \ (R' = 0.64)$$

For paint c and thickness 2.9 grams per square foot

$$D' = 0.36 \ (R' = 0.44)$$

For paint c and thickness 5.8 grams per square foot

$$D' = 0.07 \ (R' = 0.85)$$

It is obvious that values obtained on backgrounds which are darker than the indicated limits are of no interest at all. Generally, we do not know this limit. For this reason, we should assume the less favorable case. Our special curve indicates that for the determination of the obliteration of spots darker than the background, this less favorable case corresponds to a background of density $D' = 0.07$ ($R' = 0.85$) which indicates that only a white background may be tolerated. With a darker background, we run the risk of obtaining values without any relation to the paint characteristics. In every case the test must be rejected if the difference between the density value of the background and the contrast-obliterating power value is equal to the density value of the black background.

For the determination of contrast-obliterating power, referring to spots lighter than the background, Figure 6 indicates that the values for backgrounds of different reflectance change concordantly. If these two paints are representative of paints in general, it would follow that the comparison of paints as to obliteration of spots lighter than the background, on any gray (or black) background, is generally valid for every background. Since Figure 6 shows that the differences of obliteration are more pronounced on a black background than on a gray one, it is recommended that the tests be carried out on a black and not on a gray background.

All these conclusions, derived from the Kubelka-Munk theory, agree with the results of practical investigation:

The contrast-obliterating power test should primarily be carried out on a black background, thereby obtaining the most interesting values (which refer to the obliteration of spots lighter than the background).

In special cases, the results on a white background (which refer to the obliteration of spots darker than the background) may also be of interest.

The test on a gray background brings no advantage; for spots lighter than the background we obtain results which agree with those on a black background but are less exact, and for spots darker than the background, we run the risk of obtaining values which are without any relation to the paint characteristics.

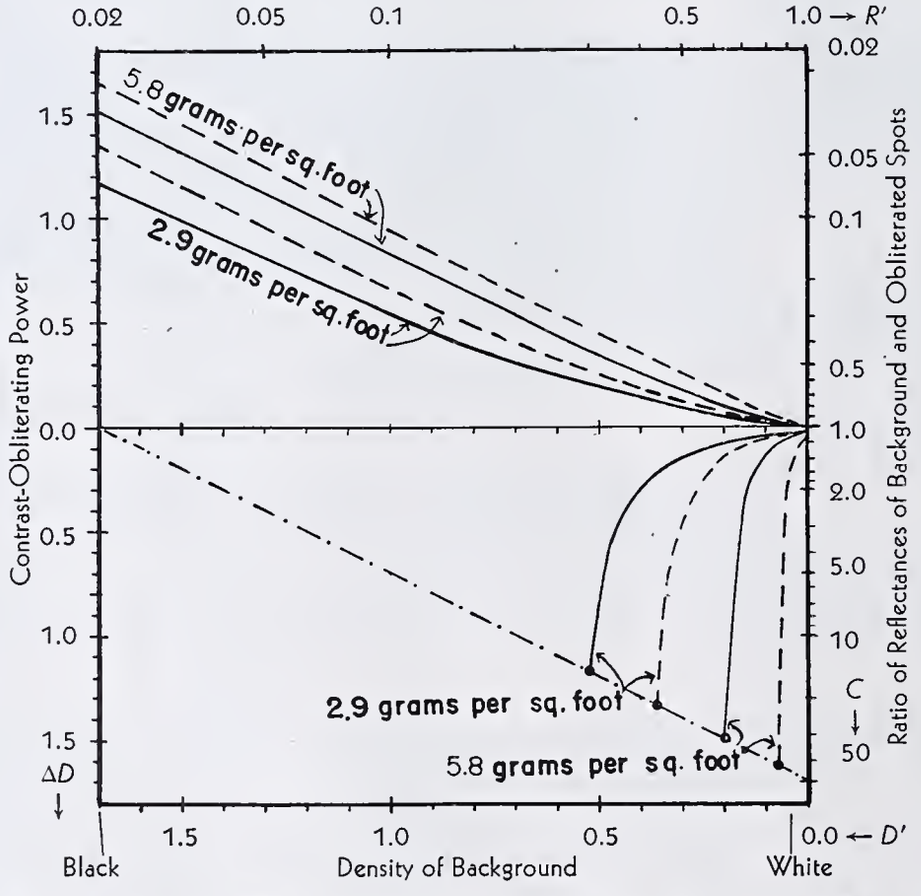

Figure 6. Contrast-Obliterating Power, Expressed as Contrast ΔD (or Reflectance Ratio C) between Background and Obliterated Gray Spot

Upper part of figure refers to spots lighter than the background ($\Delta D > 0$; $C < 1$) and lower part to spots darker than the background ($\Delta D < 0$; $C > 1$). Abscissas of intersection points of dot-dash line with curves indicate limit densities of background for obliteration of spots darker than background.

SUMMARY

The usual methods for determining hiding power of paints are not entirely satisfactory and a new method is proposed which is more closely related to practical performance of the paint.

The paint is applied in normal thickness—for example, with a Bird film applicator—on a test chart provided with a gray scale on both black and white backgrounds, and the contrast of the lightest gray shade which under these conditions is obliterated on the black background, is considered as a standard of hiding behavior, as well as the contrast of the darkest gray shade obliterated on the white background. This method is easy to carry out and independent of color and finish. No standard paint is necessary. The observations may be made on fresh or dried paint, with the naked eye and without instrumental aid. The results are obtained as absolute values indicating the (logarithmic) contrast of the obliterated spot, which is found as the difference between the densities of the background and this spot on the unpainted chart.

It is suggested that the characteristics of the paint determined in this manner be specified as contrast-obliterating power.

The practical results obtained in the determination of the contrast-obliterating

power are confirmed by a theoretical study based on the Kubelka-Munk theory.

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Nomographic Procedure for a Two-Component Colored System

Copper in a Nickel Plating Bath

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A graphical procedure is described for the calibration of a filter-type electrophotometer for a mixed-color system by measuring the relative absorption at two wave lengths. A simplified method is described for the construction of a nomograph in terms of electrophotometer scale readings. The procedure is applied to the mixed-color dithizone method for copper in a nickel plating bath.

MANY reactions giving colored products suitable for colorimetric analysis involve the treatment of two-component colored systems. The mixed color in some cases may consist of the excess colored reagent and the colored product. In other cases a foreign substance may be present which gives a colored product with the reagent. In the calibration of an electrophotometer with such a mixed-color system two general procedures have been followed; the transmittancy may be measured at one wave length in the vicinity of the maximum absorption of one of the colored components, or at two different wave lengths with the development of some relationship in the form of simultaneous equations or nomograph for the calculation. The object of this paper is to study the nomographic calculation procedure and attempt to simplify the calibration of a filter-type electrophotometer for the two-color system.

The adaptation of a monocolor method to a two-component color system offers a number of practical difficulties. Because of the broad absorption bands characteristic of most colored products, the two components invariably overlap, which makes it necessary to carry out difficult separations or to select less sensitive regions of the spectrum for the photometric measurements. If the absorption of the colored reagent occurs at the wave length selected, any variation in the concentration of the reagent will cause serious errors in the results. Unstable organic reagents which are subject to fading or oxidation are especially troublesome. Standardization of each lot of reagent is the usual procedure with this type of calibration.

The mixed-color dithizone method for copper (*8*) illustrates some of the difficulties encountered with this calibration method. Copper reacts with dithizone in dilute mineral acid solutions (0.1 *N*) to form the red-violet keto complex. The transmittancy of the mixed color may be measured at 500 to 550 mμ, the region of the maximum absorption of the copper dithizonate, or at 600 to 650 mμ, the region of maximum absorption of the dithizone (*8*). The dilute dithizone solutions customarily used (0.001 to 0.0012% by weight volume) are not very stable and tend to change on standing through evaporation or oxidation unless special precautions are taken. Since dithizone absorbs in the vicinity of 650 mμ and to a lesser extent at 525 mμ, variations in the concentration of the reagent are important. This makes it necessary to standardize each lot of reagent and make frequent checks against standards if the reagent is kept for an appreciable time. The monocolor method in which the excess dithizone is removed with ammonium by hydroxide (*1*, *3*) or pyrophosphate (*7*) has been used with this system. The difficulties encountered in the removal of the excess dithizone with ammonia are shown by Mehurin (*7*) and Sandell (*8*). The development of a calibration relationship in which the final results are independent of the initial dithizone concentration would simplify and increase the accuracy of the method.

A more permanent calibration relationship may be made by measuring the relative absorption of light in different spectral regions. This technique was applied by Kozelka and Kluchesky (*6*) to the determination of lead by the dithizone mixed-color method.

If Beer's law is obeyed, light of a given wave length from color filter A passing through a solution containing two colored components should be expressed by the relationship

$$L^A = K_1^A C_1 + K_2^A C_2 \quad (1)$$

where L^A represents the log transmittancy with filter A, and C_1 and C_2 represent the concentration of the two colored components such as, for example, dithizone and metal dithizonate. The constants K_1^A and K_2^A depend on the characteristics of color filter A, the cell thickness, and the color of components C_1 and C_2. Similarly for filter B

$$L^B = K_1^B C_1 + K_2^B C_2 \quad (2)$$

In practice only one of the concentrations of the two colored components is desired. The other colored component, usually the reagent, may vary somewhat owing to the difficulties in preparing exact concentrations of intensely colored materials but, in general, may be held within reasonable concentration limits governed by the method. If we let C_2 represent the concentration of the colored component to be measured, it follows by combining Equations 1 and 2 that $\frac{\partial L^A}{\partial C_1}$ and $\frac{\partial L^B}{\partial C_1}$ are constants. This

may be shown graphically by plotting log transmittancy L^A obtained with filter A against L^B obtained with filter B on linear graph paper as shown in Figure 1. A straight line is formed for a series of standards containing the same concentration of C_2 and variable amounts of C_1. Such a straight line will establish a relationship representing all the possible log transmittancy values of L^A and L^B for any given concentration of C_2 and variable concentrations of C_1. The C_1 concentration limits may be conveniently varied to give a suitable range for the photometric measurements.

A nomograph may be prepared from the $L^A - L^B - C_2$ relationship as shown in Figure 1. By selecting a fixed value for L^A, the L^B values may be obtained from the graph for each known concentration of C_2. A series of lines may be drawn in this manner, giving a nomograph similar to the one given in Figure 2. Interpolation may be used for the values of L^A within 1 or 2 scale division steps used in preparing the chart.

Kozelka and Kluchesky (*6*) obtained this relationship by adding measured volumes of dithizone solution to the standard lead dithizonate. Constants K are calculated from the electrophotometric values of L^A. Owing to the change in concentration of the metal complex brought about by the change in volume of the organic solvent, Taylor's expansion must be employed to correct the values for the change in C_2. This procedure is somewhat complicated and assumes no change in the log transmittancy of the metal dithizonate at 660 mμ, which may not always be true. A simple procedure which eliminates this difficulty would be to hold the volume of the organic solvent layer constant and obtain variations in the dithizone concentration by dilution with carbon tetrachloride. A stronger dithizone solution may be used and the sum of the volume of dithizone solution plus the solvent used for dilution may be held at a fixed value, such as, for example, 10 ml. Four or five separate standards prepared in this manner should suffice to establish a well-defined straight line for the $L^A - L^B$ relationship for each increment of copper. A series of lines is shown in Figure 1. Such a series of lines represents all the possible combinations of L^A and L^B in the most useful range of dithizone–copper dithizonate concentrations. From this relationship obtained with the two color filters a nomograph may be prepared for any particular value of L^B, as in Figure 2.

Another possible simplification is the establishment of the relationship given in Equations 1 and 2 in terms of electrophotometer scale rather than log transmittancy values. This simplifies the calculation and the use of the nomograph given in Figure 2. The log scale on the Fisher electrophotometer has been described (*2*).

Copper is one of the impurities frequently encountered in a nickel plating bath. Copper is especially objectionable in bright nickel plating, since darkening of the deposit may occur at low current density areas. Accordingly, some rapid method for the estimation of small amounts of copper would be useful in the control of the plating operations. The mixed-color dithizone method with the extraction of the copper as copper dithizonate from 0.1 N hydrochloric acid solution has been used by Knapp (*5*) for this purpose. Nickel does not form a colored complex at a pH of 1.3 to 2.3 and small amounts of copper from 0.001 to 0.05 gram per liter may be separated from the nickel without interference. This separation eliminates the well-known sulfide separation which has been shown to offer a number of difficulties (*4*).

MATERIALS AND REAGENTS

Electrophotometer. The AC Model Fisher electrophotometer equipped with 5-mm. test-tube type cells, 525 and 650 mμ color filters, was used.

Hydrochloric Acid, approximately 0.1 N. Dilute 8.9 ml. of reagent grade acid, specific gravity 1.18, to 1000 ml. with redistilled water.

Carbon Tetrachloride. Best grade analytical reagent quality free from impurities that inhibit the reaction between the dithizone and copper should be used. Check each lot for extractability by noting the shaking time required to develop the maximum color. Mallinckrodt's analytical reagent grade was found satisfactory without distillation.

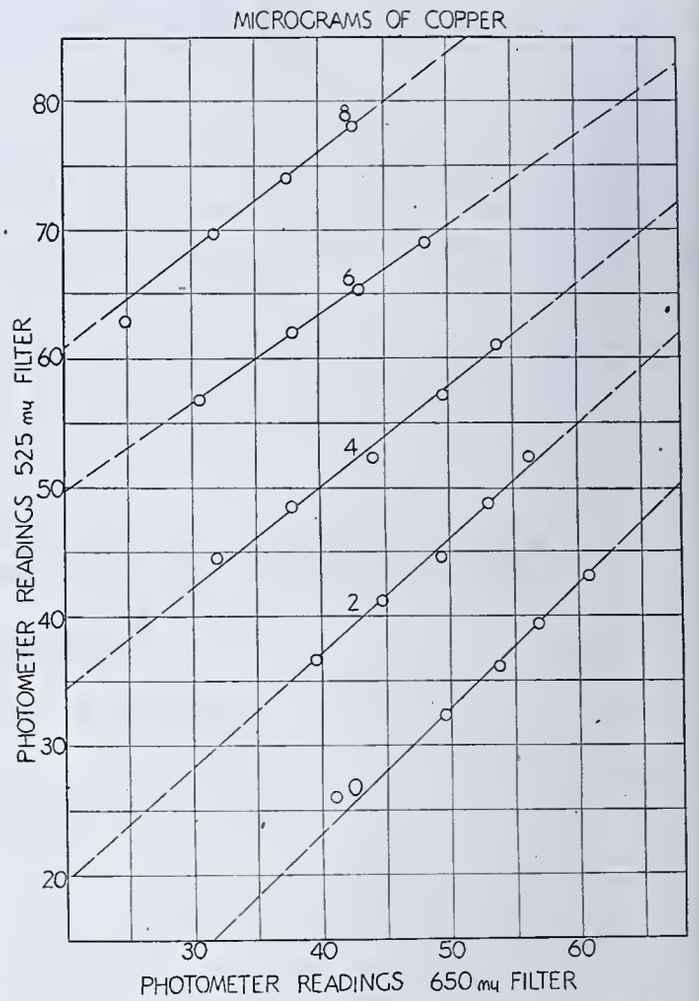

Figure 1. Photometric Relationship at 525 and 650 mμ

Dithizone Solution. For the calibration dissolve 12 mg. of diphenylthiocarbazone in 1 liter of carbon tetrachloride. For the method dissolve 1 to 1.2 mg. in each 100 ml. of carbon tetrachloride. Store at 5° C. and prepare fresh each week. Dithizone may be obtained commercially in a high state of purity or recrystallized if necessary.

Copper Standard. Dissolve 0.1000 gram of electrolytic copper foil in dilute nitric acid and dilute to 1000 ml. Pipet 10 ml. into a 1000-ml. volumetric flask, add 8.9 ml. of hydrochloric acid, and dilute to 1000 ml.

PROCEDURE AND APPLICATION

Calibration. Prepare a series of four or five standards containing the same copper concentration by taking a measured volume of copper standard in 125-ml. separatory funnels and sufficient 0.1 N hydrochloric acid to make the volume 25 ml. Add 10 ml. of dithizone solution to one separatory funnel, 9 ml. of dithizone plus 1 ml. of carbon tetrachloride to the second, 8 ml. of dithizone plus 2 ml. of carbon tetrachloride to the third, and so forth. Shake vigorously for 3 minutes. If a purple color is developed shake for 2 additional minutes. Filter the chloroform layer into the absorption cells through a small pledget of cotton to remove droplets of the aqueous layer. Measure the A scale or log scale, reading the electrophotometer with the 525 and 650 mμ color filters, using an absorption cell filled with carbon tetrachloride at 0 scale as a reference standard.

Repeat this procedure for each increment of copper. Five different copper concentrations should suffice for the construction of the nomograph. Plot the 525 mμ electrophotometer readings as ordinate against the 650 mμ readings as abscissa on linear graph paper. A series of straight lines should be formed, as in Figure 1.

Prepare a nomograph by obtaining from Figure 1 the electrophotometer values for the 525 mμ photometer reading for a given 650 mμ value. Plot the values for 0, 2, 4, 6, and 8 micrograms of copper for each 2 divisions on the 650 mμ values.

To use the nomograph select the particular line corresponding to the 650 mμ photometer value and determine the intersection of this line with the 525 mμ value. Read the copper concentration from the abscissa axis. For example, a photometer reading

of 36 at 650 mμ and 60 at 525 mμ would indicate a copper concentration of 6 micrograms.

METHOD. Pipet a 10-ml. sample of a nickel plating solution into a 100-ml. volumetric flask, and dilute to the mark with 0.1 *N* hydrochloric acid. Pipet a suitable aliquot from 1 to 10 ml. into a 125-ml. pear-shaped separatory funnel. Adjust the volume to 25 ml. with 0.1 *N* hydrochloric acid. Add exactly 10 ml. of dithizone solution and shake vigorously for 3 to 5 minutes. Allow the layers to separate and filter the colored carbon tetrachloride layer through a small pledget of cotton into a photometer cell. Measure the transmittancy against the carbon tetrachloride as a reference with the 525 and 650 mμ color filters, respectively. Refer to the nomograph for the copper concentration.

RESULTS. Known amounts of copper were added to a sample of a Watts type nickel plating solution containing 240 grams per liter of nickel sulfate hexahydrate, 60 grams per liter of nickel chloride hexahydrate, and 45 grams per liter of boric acid with a pH of 3.0. Traces of copper found in the salts were removed by extraction at pH 2 with 0.01% (weight volume) dithizone solution in carbon tetrachloride followed by washing with pure carbontetrachloride. The copper was then determined on a suitable aliquot by the mixed color procedure given in the method. The results obtained are given in Table I.

Table I. Recovery of Copper Added to a Nickel Plating Solution

Sample Size	Copper Added		Copper Found	Difference
Ml.	γ	*G./l.*	*G./l.*	*G./l.*
1	0.0	0.0000	0.0001	+0.0001
	0.0	0.0000	0.0002	+0.0002
	0.8	0.0008	0.0010	+0.0002
	1.8	0.0018	0.0019	+0.0001
	2.4	0.0024	0.0026	+0.0002
	6.4	0.0064	0.0064	±0.0000
	7.4	0.0074	0.0076	+0.0002
	8.0	0.0080	0.0079	−0.0001
0.1	0.4	0.004	0.005	+0.001
	1.6	0.016	0.016	±0.000
	2.4	0.024	0.022	−0.002
	3.2	0.032	0.033	+0.001
	4.4	0.044	0.043	−0.001
	5.6	0.056	0.054	−0.002

DISCUSSION

The advantages in the establishment of a relationship given in the nomograph for a relatively wide range of dithizone concentration are readily apparent. The dithizone carbon tetrachloride solution may be prepared in small quantities as needed, with the approximate concentrations governed by the calibration. In this manner the difficulties due to the oxidation of the reagent or evaporation of the solvent in storage are avoided. Checking the calibration curve at frequent intervals is made unnecessary. The errors due to the oxidation of the dithizone to a colorless keto form are minimized by using freshly prepared solutions. Little change due to oxidation of the reagent was observed over a 1-week period.

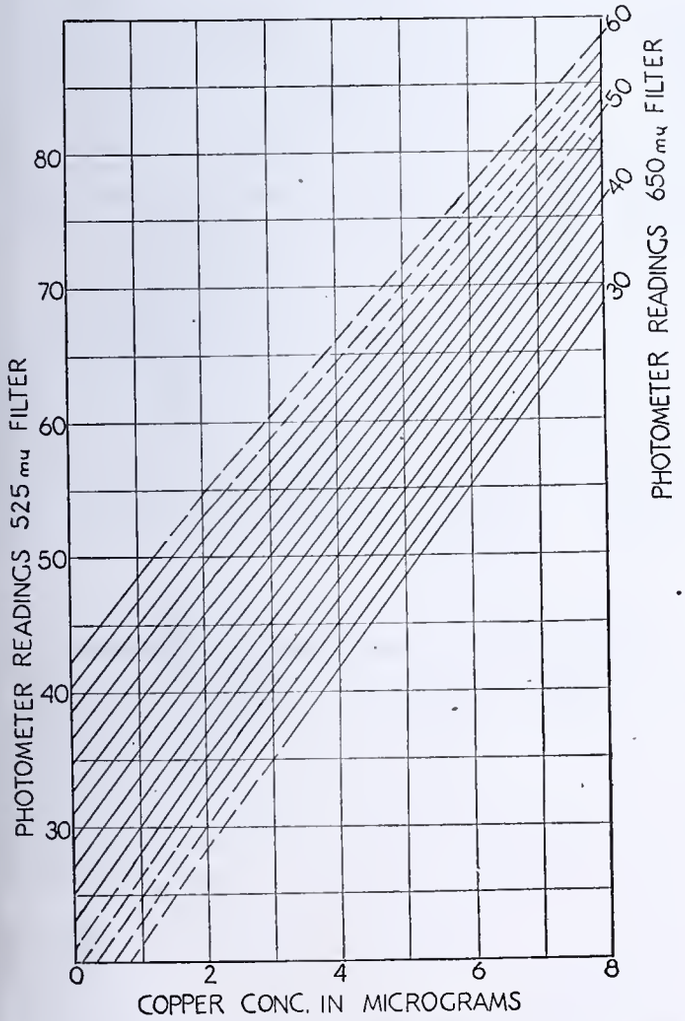

Figure 2. Nomograph for the Dithizone–Copper Dithizonate System

Care should be exercised in keeping the $L^A - L^B$ values within the limits of the experimental values given in Figure 1, because of possible deviations from Beer's law with greater differences in concentrations. This may be conveniently done by the use of dotted lines in the nomograph to cover values outside the limits of the experimental data. A rough check made by measuring the transmittancy of the original dithizone solution with the two filters, and referring to the nomograph will aid in governing the proper concentrations.

Bendix and Grabenstetter (*1*) found a shaking period of 10 minutes necessary in order to obtain an equilibrium between the copper in the acid aqueous solution and the copper dithizonate in the carbon tetrachloride solution. An examination of several lots of reagent grade carbon tetrachloride showed some variation in the extractability of the copper dithizonate. Four out of five lots examined gave a maximum color development with 3 to 5 minutes' shaking. One lot required 7 minutes to establish an equilibrium. Sandell (*8*) suggests the presence of traces of impurities, probably sulfur compounds, which tend to inhibit the reaction between copper and dithizone in acid solution. Since serious errors may be caused through failure to develop the equilibrium condition with an insufficient shaking period, each lot of carbon tetrachloride must be examined for extractability by noting the time required to develop a maximum color. No difficulty was experienced in obtaining carbon tetrachloride which developed a maximum color within 3 to 5 minutes' shaking. The addition of a small amount of a wetting agent such as 2 drops of a 1% aqueous solution of aerosol OT (a product of the American Cyanamid Company) tends to improve the extractability without disturbing the equilibrium value.

Nickel in concentrations up to 78 mg. does not seriously interfere with the estimation of copper without a preliminary separation. The results in Table I show the interference to be less than 0.2 microgram. Interfering metals that form colored dithizonates from dilute acid solutions, such as mercury, palladium, gold, and silver, are not likely to be present in a nickel plating bath; however, interference may be expected if they are present.

The calibration procedure described could be adapted to other mixed-color procedures. The well-known dithizone methods for the heavy metals are merely examples of the general nomographic calibration method. The procedure is by necessity limited to a two-component colored system.

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Differential Reduction of Iron and Tin

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A method is described for the titrimetric determination of iron and tin on the same weighed sample of material. The method is suitable for bronze and other copper alloys containing less than 1% antimony. After separation from copper, the iron is reduced in an inert atmosphere with metallic nickel in cold dilute hydrochloric acid and titrated with potassium dichromate solution. Following this, the tin is reduced with metallic nickel in the hot, concentrated acid, cooled under an inert atmosphere, and titrated with iodine solution. The method requires 1.5 hours for a determination and is suitable for routine analysis.

THE method described herein was developed to overcome the usual difficulties encountered in the determination of iron in bronze and similar alloys containing tin. In the nitric acid separation of tin as metastannic acid, considerable iron is brought down in the precipitate. For this reason, the often described procedure of separating iron as the hydroxide, after prior removal of tin, lead, and copper in nitric acid solution, will lead to low results in the determination of iron. This error is often neglected in routine analysis, on the assumption that it is small. It has been found in this laboratory that as much as one half of the total iron present may be held in the tin precipitate—i.e., in a sample containing 0.0056 gram of iron and approximately 0.075 gram of tin, 0.0023 gram of iron was found in the metastannic acid precipitate, after careful washing.

Of the available methods for recovery of this iron from the tin precipitate, the use of hot ammonium sulfide is perhaps the most satisfactory. A procedure involving ignition of the metastannic acid, volatilization of the tin by the use of ammonium iodide as in the method of Caley and Burford (*1*), and recovery of iron from the residue has also been used with success. Both methods are time-consuming and unpleasant to use. In the presence of precipitated tin, efforts to determine the iron by titration with potassium dichromate, using a modified Knop's (*2*) procedure, were only partially successful.

It is customary, in this laboratory, to determine tin by the method of McDow, Furbee, and Clardy (*3*). The tin is collected by precipitation as hydroxide in ammoniacal solution, using aluminum hydroxide as a collector to ensure complete precipitation of the tin. Iron is also completely precipitated at this point and it was desirable to use this sample for the determination of both iron and tin.

Since tin was subsequently to be determined, the reduction of which necessitated a fairly concentrated solution, it was desirable that the iron be reduced by a method that would cause no loss of tin and would not increase the volume materially, and where the reagent used would be without effect on the titration of iron and tin or be easily removable.

It was found that nickel in cold dilute hydrochloric acid solution would effectively reduce iron without causing appreciable reduction of tin. Since this same reagent in hot concentrated acid is effective for the reduction of tin and it meets the requirements outlined above, it appeared that nickel could be made the basis for a method for the determination of both tin and iron. This assumption has been verified by subsequent investigation.

REAGENTS REQUIRED

ALUMINUM NITRATE. Dissolve 10 grams of aluminum nitrate, C.P. reagent, in 100 ml. of water.

POTASSIUM DICHROMATE. Stock Solution. Dissolve 87.81 grams of C.P. potassium dichromate, $K_2Cr_2O_7$, in water and dilute to 1000 ml.

Standard Solution. Dilute 10 ml. of the stock solution of potassium dichromate to 1000 ml. with water. Standardize against C.P. iron. One milliliter of the potassium dichromate solution is approximately equivalent to 0.001 gram of iron.

MERCURIC CHLORIDE. A saturated solution of mercuric chloride in water.

SODIUM DIPHENYLAMINE SULFONATE. This indicator is prepared by the method of Willard and Young (*4*). Dissolve 3.2 grams of C.P. barium diphenylamine sulfonate in 1000 ml. of water, add an excess of sodium sulfate, and filter.

NICKEL, C.P. nickel shot, less than 6 mm. in diameter.

STANDARD IODINE SOLUTION. Dissolve approximately 11 grams of iodine and 20 grams of potassium iodide in 100 ml. of water and dilute to 1000 ml. Standardize against pure tin, using the method of reduction described in the text.

STARCH SOLUTION. Prepare a thin paste consisting of 5 grams of soluble starch, 10 grams of sodium bicarbonate, and water. Pour the paste into 300 ml. of boiling water, and boil for a minute or two, with stirring. Cool the solution and dilute to 1000 ml.

PHOSPHORIC-SULFURIC ACID REAGENT. Mix carefully 800 ml. of water, 100 ml. of sulfuric (sp. gr. 1.84), and 100 ml. of phosphoric (sp. gr. 1.69) acids.

METHOD

Weigh a quantity of sample, preferably containing not more than 0.10 gram of tin and not more than 0.05 gram of iron, into a 300-ml. Erlenmeyer flask. Disregarding the formation of metastannic acid, add sufficient dilute nitric acid (1 to 1) to dissolve the metal. Boil to expel oxides of nitrogen, dilute with water to approximately 100 ml., and add 3 to 5 ml. of aluminum nitrate solution. Cautiously add ammonium hydroxide until the blue copper complex forms, then add 10 ml. in excess. Boil gently to coagulate the mixed precipitate of iron, aluminum, and tin hydroxides. Filter and retain the precipitate on Whatman No. 42 or equivalent filter paper, washing four or five times with 2% ammonium hydroxide to remove the copper salts.

Return the filter paper and precipitate to the original flask. Add 10 ml. of perchloric acid (sp. gr. 1.67) and 5 ml. of nitric acid (sp. gr. 1.42) and heat gently to destroy the filter paper. (To avoid danger of explosion, nitric acid should be added dropwise if solution turns dark or black.) Dilute to 100 ml., reprecipitate with excess ammonia, filter, and wash precipitate thoroughly. Destroy paper as before but add 10 ml. of sulfuric acid (sp. gr. 1.84).

When the organic matter is destroyed, evaporate solution over an open flame to the appearance of sulfur trioxide fumes. Allow to cool.

Table I. Effect of Temperature on Reduction of Tin

Tin Present	Temperature	Time Shaken	Tin Reduced
Gram	*° C.*	*Min.*	*%*
0.06	6	15	20
0.06	12	15	25
0.06	17	15	30
0.06	23	15	60

Table II. Effect of Tin Concentration on Reduction of Tin

Tin Present	Temperature	Time Shaken	Tin Reduced
Gram	*° C.*	*Min.*	*%*
0.10	6	15	75
0.06	6	15	20
0.02	6	15	Trace
0.01	6	15	Trace

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[2] Present address, 595 Elizabeth Ave., Newark, N. J.

Table III. Analyses of Alloys of High Percentage Tin

(Determination of blank)

Bureau of Standards Sample No.	No. of Analyses	% Iron Present	% Iron Found	% Iron Difference
124a (4.82% tin)	6	0.004	0.027	0.023
52b (8.00% tin)	6	0.032	0.057	0.025
52 (7.89% tin)	3	0.12	0.14	0.02
63 (9.91% tin)	3	0.28	0.30	0.02
63a (9.76% tin)	3	0.52	0.55	0.03
Synthetic Standards				
A (124a + 0.05% iron)	1	0.054	0.073	0.019
B (124a + 0.10% iron)	1	0.104	0.127	0.023
C (52b + 0.05% iron)	3	0.082	0.105	0.023
D (52b + 0.10% iron)	3	0.132	0.157	0.025
E (5.00% tin)	2	0.10	0.12	0.02
F (5.00% tin)	2	0.50	0.51	0.01
G (5.00% tin)	3	1.00	1.01	0.01

Average difference (36 samples), 0.02%.

DETERMINATION OF IRON

Cautiously dilute contents of flask with water to a volume of 50 ml., add 15 ml. of concentrated hydrochloric acid, and boil until solution is clear. Remove to water bath. Add solid carbon dioxide. Cool below 20° C. in ice bath, add approximately 30 grams of small nickel shot, close flask with rubber stopper as last lumps of carbon dioxide disappear, and shake vigorously on a mechanical shaker for 15 minutes. Chill again in ice bath and decant the solution into a 500-ml. vial-mouth extraction flask. Wash the nickel shot twice by decantation with small portions of cold water. Reserve the nickel shot for the tin determination.

To the reduced iron solution add 10 ml. of mercuric chloride solution, 3 drops of sodium diphenylamine sulfonate as indicator, and 20 ml. of the sulfuric-phosphoric acid reagent. Titrate the solution with standardized potassium dichromate to the appearance of a purple end point which persists. In high tin alloys a blank correction of 0.02% must be subtracted.

Return the reserved nickel shot to the solution, add 60 ml. of hydrochloric acid, and proceed with the tin determination.

DETERMINATION OF TIN

The method used for the determination of tin was substantially as described by McDow, Furbee, and Clardy (*3*).

The apparatus was modified to make use of a 500-ml. vial-mouth extraction flask and consisted of a heating element, the extraction flask, a one-hole No. 10 rubber stopper, and a glass tube suitably bent so that it extended from the flask to a beaker containing water or the sodium bicarbonate solution.

The 500-ml. extraction flask, containing the tin solution, nickel shot, and hydrochloric acid, is closed with the rubber stopper and the exhaust tube is placed in a beaker of water. The solution in the flask is brought rapidly to boiling and the reaction allowed to proceed for 0.5 hour at low heat. The glass tube is then placed in a beaker containing 10% sodium bicarbonate solution, the flask is removed from the heater, and the contents are cooled below 20° C. in an ice bath. The stopper is removed and a small piece of dry ice rapidly added.

The solution of stannous tin is titrated with standardized iodine solution, under an atmosphere of carbon dioxide, to a permanent dark blue color change, using 5 ml. of starch solution as indicator.

DISCUSSION

Table I shows the effect of temperature on the reduction of tin. With increasing temperature, increasing amounts of tin are reduced. The reduction of a small amount of tin is desirable, as it is evidence that all the iron is in the ferrous state. In alloys containing a large amount of tin this reduction should be minimized by cooling, so that the amount of mercuric chloride will be ample to affect its purpose.

Table II shows the per cent of tin reduced in an inert atmosphere by shaking with nickel shot. As the concentration of tin increases, the percentage reduction becomes greater.

Table III establishes a correction factor when high-percentage tin alloys are encountered. This blank of 0.02% is presumably due to the increased amount of mercurous chloride because of the reaction of the larger amount of stannous salts with mercuric chloride.

Table IV establishes the accuracy of the method in simple bronzes and brasses, manganese bronze, phosphor bronze, and samples containing up to 1% antimony. (Pure samples of antimony, 0.01, 0.05, and 0.10 gram, which were treated according to the prescribed procedure, required 0.1, 0.4, and 1.3 ml. of the titrating solution, respectively.) Out of fifty-five analyses the maximum deviation was less than ±0.02% on samples containing as much as 1% iron.

Table IV. Determination of Iron and Tin

Bureau of Standards Sample	% Iron No. of Detns.	% Iron Max. deviation	% Iron Present	% Iron Found	% Tin No. of Detns.	% Tin Max. deviation	% Tin Present	% Tin Found
Sheet brass No. 37	3	0.00	0.29	0.29	4	0.02	1.01	1.00
Sheet brass No. 37b	4	0.01	0.21	0.21	3	0.01	0.99	1.00
Manganese bronze No. 62	3	0.01	1.13	1.13	4	0.01	0.82	0.81
Manganese bronze No. 62a	3	0.01	1.04	1.04	4	0.01	0.84	0.83
Manganese bronze No. 62b	4	0.02	0.82	0.82	4	0.00	0.97	0.97
Effect of Antimony								
Cast bronze No. 52 (0.16% Sb)[a]	3	0.00	0.12	0.12	2	0.03	7.90	7.89
Phosphor bronze No. 63 (0.55% Sb)[a]	3	0.01	0.27	0.28	3	0.02	9.91	9.91
Phosphor bronze No. 63a (0.49% Sb)[a]	3	0.01	0.52	0.53	2	0.04	9.76	9.74
Synthetic Samples								
Sample A (0.20% Sb)	2	0.02	0.50	0.49	.	..	..	..
Sample B (0.50% Sb)	2	0.01	0.50	0.50	.	..	..	..
Sample C (1.00% Sb)	2	0.01	0.50	0.50	.	..	..	..
Effect of Tin								
Sample D (0.00% Sn)	2	0.01	0.10	0.11	.	..	..	..
Sample E (0.00% Sn)	2	0.02	0.50	0.49	.	..	..	..
Sample F (0.00% Sn)	4	0.02	1.00	0.99	.	..	..	..
Sample G (1.00% Sn)	2	0.01	0.10	0.11	.	..	..	..
Sample H (1.00% Sn)	2	0.01	0.50	0.51	.	..	..	..
Sample I (1.00% Sn)	4	0.01	1.00	1.00	.	..	..	..
Sample J (5.00% Sn)[a]	2	0.02	0.10	0.10	.	..	..	..
Sample K (5.00% Sn)[a]	2	0.01	0.50	0.49	.	..	..	..
Sample L (5.00% Sn)[a]	3	0.02	1.00	0.99	.	..	..	..

[a] See Table III.

PRECAUTIONS

The final fuming of the salts to sulfuric acid fumes is essential. Discordant results are obtained if this is not accomplished.

An inert atmosphere is necessary for complete reduction, but caution is advised in stoppering the flask before all the lump carbon dioxide is evolved.

The nickel shot should be cleaned by boiling for a few minutes in hydrochloric acid. It will brighten on repeated use, probably because of amalgamation with the mercury in the mercuric chloride.

The complete reduction of the iron depends upon vigorous mechanical shaking because of the small surface presented by the nickel shot. With samples containing a high percentage of tin, the time required for shaking can be reduced if desired.

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Detection of Elemental Sulfur in Gasoline by the Sommer Test

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The sensitivity of various tests for the detection of elemental sulfur in gasoline has been determined; and a modification of the Sommer test is described which is about five times as sensitive as the customary inverted doctor test using butyl mercaptan.

SOMMER (*1*) describes a color test for free sulfur in pyridine solution, in which the addition of a small amount of an alkaline solution precipitates the sulfur as a blue colloidal solution. A positive reaction was obtained with as little as 2 parts per million of free sulfur; but hydrocarbons were observed to decrease the sensitivity of the test. In the presence of pyridine, doctor solution gave a visible reaction with 10 micrograms of free sulfur per cubic centimeter (10 p.p.m. on w/v basis).

Sommer's test was applied to the detection of free sulfur in gasoline by mixing the sample with pyridine and adding caustic soda solution. In the test, three phases were formed: an upper gasoline phase, a middle pyridine phase, and a lower aqueous phase. The color appeared in the middle pyridine phase, occasionally in the lower aqueous phase, but never in the upper gasoline phase. A preliminary survey showed that this test was at least as sensitive as the inverse doctor test, using butyl mercaptan solution. This is customarily used in plant testing but has, at times, appeared to be insufficiently sensitive.

The alternative mercury test is generally considered too sensitive for plant control.

The conditions for the maximum sensitivity of the Sommer test were determined, and the sensitivity of the various tests was compared.

EXPERIMENTAL

REAGENTS USED. Gasoline. For the purposes of this investigation, except as noted below, commercial iso-octane of specific gravity 0.691, freed of possible traces of sulfur and filtered before use, was used as gasoline and is referred to as such. The commercial iso-octane, as received, was shaken with mercury to remove possible traces of free sulfur and filtered before use. Only a negligible amount of mercuric sulfide was formed.

Standard Sulfur Solution, prepared by dissolving flowers of sulfur in 1 cc. of hot pyridine and allowing the solvent to evaporate almost to dryness before adding sufficient gasoline to give a solution containing 0.01% of sulfur by weight. Other concentrations were obtained by diluting the original standard with gasoline.

Pyridine. A water-white, commercial grade of pyridine containing approximately 5% of alpha-picoline was used untreated as it was not discolored on shaking with mercury.

Butyl Mercaptan Solution, 1 cc. of *n*-butyl mercaptan dissolved in 1400 cc. of gasoline, thoroughly shaken with mercury, and filtered before use. This treatment satisfactorily removed any free sulfur present, and did not affect the mercaptan content of the solution.

Doctor Solution, 10% caustic soda solution shaken with excess litharge for a quarter of an hour, allowed to settle, and the clear supernatant liquor decanted for use.

Caustic Soda Solution, 2 *N*.

Sodium Bicarbonate Solution, cold saturated.

SOMMER TEST. The necessary conditions for the test to give maximum sensitivity were obtained as follows:

Alkaline Solution. Sommer used 2 *N* caustic soda and saturated sodium bicarbonate solutions and obtained greater sensitivity with the latter. However, in the presence of gasoline, the caustic soda solution was found to give several times the sensitivity of the sodium bicarbonate solution, which also required boiling to develop the color. The caustic soda solution was therefore used.

Order of Mixing. The addition of the caustic soda solution to the pyridine-gasoline mixture gave a color several times as intense as that produced by the addition of the pyridine to the gasoline–caustic soda mixture.

Pyridine-Gasoline Ratio. Various volumes of a 0.001% (w/w) solution of free sulfur in gasoline were added to 5 cc. of pyridine and 1 cc. of 2 *N* caustic soda solution was added and shaken. The results were:

Test positive	2 to 20 cc. of gasoline
Test strongly positive	3 to 15 cc. of gasoline
Test maximum color	7 to 9 cc. of gasoline

Pyridine–Caustic Soda Ratio. Different volumes of 2 *N* caustic soda solution were added to a mixture of 3 cc. of pyridine and 5 cc. of 0.001% (w/w) solution of sulfur in gasoline. The test was definitely positive with up to 1.6 cc. and questionable with 2.0 cc. of caustic soda solution. The maximum color was obtained with 0.3 cc., though there was very little difference between the colors produced with 0.3 to 0.6 cc. The maximum was obtained with the same pyridine–caustic soda ratio (10 to 1) as used by Sommer.

Nature of Gasoline. The conditions of the test (5 cc. of gasoline, 3 cc. of pyridine, and 0.5 cc. of 2 *N* caustic soda solution) and the sensitivity were unchanged with 100% cracked gasoline (from the thermal cracking of shale oil).

No highly aromatic gasoline was available, so mixtures of motor benzene and commercial iso-octane were examined. It was found that the proportion of the middle pyridine phase decreased as the benzene content of the blend increased but the sensitivity of the test appeared to be unchanged. Sixty per cent of benzene in the blend was the maximum that could be tested without changing the conditions of the test. Increasing the amounts of pyridine and caustic soda and decreasing the caustic soda concentration enabled the test to work satisfactorily with higher benzene blends:

Benzene in Blend	Volume of Blend	Volume of Pyridine	Caustic Soda Solution
%	*Cc.*	*Cc.*	*Cc.*
60	5	4	1.5 2 *N*
70	5	5	2 2 *N*
80	5	5	2 *N*

Blends containing much more than 80% benzene did not give three phases in the test, though a blue color was developed in the pyridine-gasoline phase. The sensitivity of the test was, however, much reduced.

With the benzene blends, the blue color was less stable to air and was destroyed by shaking for a minute or so.

TESTS COMPARED FOR SENSITIVITY. In order to compare the relative sensitivity of the different tests available, the following tests were carried out:

Inverse Doctor Test. Five cubic centimeters of the sulfur-containing gasoline were shaken with 5 cc. of butyl mercaptan solution and 5 cc. of doctor solution. In the absence of free sulfur, the lead butyl mercaptide formed colored the gasoline phase yellow and some yellow mercaptide collected at the interface. In the presence of free sulfur, the color deepened through orange to dark brown or even black, depending on the concentration. Owing to the color of the mercaptide, it was necessary to use a blank test for comparison with borderline tests.

Modified Inverse Doctor Test. Five cubic centimeters of the sulfur-containing gasoline were mixed with 5 cc. of pyridine and 5 cc. of butyl mercaptan solution before addition of 5 cc. of doctor solution. The addition of the pyridine prevented the color formation due to the lead mercaptide. Three liquid phases were formed and, in the presence of sufficient free sulfur, the lead sulfide formed collected mainly at the liquid phase interface, making this test easier to observe than the unmodified test.

Sommer Test. Five cubic centimeters of the sulfur-containing gasoline were mixed with 3 cc. of pyridine and then shaken with 0.5 cc. of 2 *N* caustic soda solution. In the presence of free sulfur, a sky-blue coloration was formed in the middle phase. On long shaking in the presence of air, the color disappeared, this being due to the oxidation of the colloidal sulfur to thiosulfate.

Mercury Test. Five cubic centimeters of the sulfur-containing gasoline were shaken with a little mercury. In the presence of free sulfur, black mercury sulfide was formed and the suspension colored the gasoline gray.

OTHER APPLICATION OF TESTS. Attempts to use purified pyridine homologs (boiling range 120° to 250° C.), isolated from the racked pressure distillate, in place of pyridine in the Sommer est failed. This was due to the higher miscibility of these bases ith the gasoline, and the failure of the mixture to separate into hree phases on the addition of water. This is probably the esensitizing action of hydrocarbons on the test that was observed by Sommer. An attempt was made to apply these different tests to detect free sulfur in crude shale oil, but alkali-soluble oloring matter in the oil masked any color reactions.

RESULTS

The results are expressed in sulfur concentration in parts per illion by weight required to give a definite, questionable, or egative test. With the standard solutions used, 1 p.p.m. (w/w) s equal to 0.7 microgram per cubic centimeter.

Test	Definite	Questionable	Negative
	P.p.m.	*P.p.m.*	*P.p.m.*
nverse doctor test	20	15	10
nverse, modified by presence of pyridine	15	10	8
ommer test	4	3	2
Iercury test	Positive even below 0.1 p.p.m.		

The mercury test is by far the most sensitive, but it is too ensitive for plant control. The inverted doctor test is positive to 15 p.p.m. of free sulfur, but the color of the lead mercaptide tends to make detection difficult near the limiting concentration; in the presence of pyridine, the modified test is easier to observe and a shade more sensitive. The Sommer test is sensitive to 3 p.p.m. of free sulfur in the gasoline, being therefore about five times as sensitive as the inverted doctor test, and yet not too sensitive for plant control.

With experience, a plant operator is able to obtain an approximate estimate of the free sulfur content of the gasoline from any of the first three tests, but much more readily from the Sommer test. The Sommer test has been found both quicker to operate and easier to observe than the inverse doctor test previously used.

If the gasoline being tested is highly aromatic, it would be advisable to determine the optimum proportions of the reagents for the test before putting it into routine operation.

ACKNOWLEDGMENTS

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Qualitative Test for Carbohydrate Material

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A solution of anthrone in concentrated sulfuric acid gives a permanent green coloration with carbohydrate material. The reaction is of value as a qualitative test and for the preliminary classification of synthetic resins into a cellulose or noncellulose group.

ALTHOUGH there are a large number of specific tests for certain types of saccharides (*1*, *2*), there are very few general tests for carbohydrate materials. The Molisch test, using α-naphthol, is well known and generally applicable to soluble carbohydrates.

Anthrone, which is used for the determination of glycerol (*4*), was found to give a green color with cellulose. Further experiments indicated that a positive test was obtained with all of a group of eighteen carbohydrate materials examined, including several cellulose derivatives. Furfural is the only noncarbohydrate material, thus far encountered, which gives a green color with anthrone. The test as given by furfural is, however, different from that given by carbohydrate materials. The green color given by a furfural test is rapidly obscured by a brown precipitate, and when the sample is diluted with 50% sulfuric acid or glacial acetic acid, a heavy brown precipitate forms. Carbohydrate samples, on the other hand, may be diluted to any extent with these reagents, and the green color persists even at extreme dilutions.

Positive Test with Anthrone

Cellulose	Ethylcellulose (ether)
Starch	Methylcellulose (ether)
Dextrin	Cellulose acetate
Dextrose	Cellulose acetate phthalate
l-Arabinose	Cellulose acetate butyrate
Gum arabic	Cellulose acetate stearate
Gum tragacanth	Cellulose propionate
Agar	Cellulose nitrate
Pectin	Furfural (noncarbohydrate)
Algin	

A negative test was obtained by all of a large group of noncarbohydrate materials examined, which included a variety of noncellulose synthetic resins, organic acids, aldehydes, phenols, fats, terpenes, alkaloids, and proteins.

PROCEDURE

One milliliter of water is placed in a small test tube containing approximately 1 mg. of the material to be tested, and 2 ml. of a 0.2% solution of anthrone in concentrated sulfuric acid are then added. The final sulfuric acid concentration in the test solution should always be greater than 50%; otherwise the anthrone will come out of solution and produce a milky suspension. The heat produced by the dilution of the sulfuric acid is a necessary part of the test. In the presence of carbohydrate material a clear green color will appear and rapidly increase in intensity until a dark blue-green solution results. The test solution can be diluted for comparison with glacial acetic or 50% sulfuric acid. In the absence of carbohydrate material, but in the presence of other organic compounds, a brown color is often produced by the action of the concentrated sulfuric acid.

The anthrone, which is not a readily available chemical, can be prepared according to the directions of Schutz (*4*), or following the procedure given in Organic Syntheses (*3*). Care should be taken during the preparation to avoid contamination of the anthrone by carbohydrate material, especially filter paper pulp, which would cause a green color to develop in a blank test with the reagent.

DISCUSSION AND APPLICATIONS

No study was made of the mechanism of the reaction, but the appearance and deepening of the color seemed to be as rapid with a polysaccharide as with an equal weight of a monosaccharide. This would suggest that hydrolysis may not be a necessary step in the test.

A useful application of this reaction is the identification of synthetic resins. Even the most insoluble cellulose resins will give a positive test with anthrone, thus affording a preliminary classification into a cellulose or noncellulose group. However, many plastic molding compositions contain wood flour and would give a positive test for that reason.

An attempt is now being made to apply this reaction to the quantitative colorimetric determination of small quantities of cellulose in solution, particularly to the analysis of β- and γ-cellulose.

The anthrone test is extremely sensitive. In tests with starch it proved to be 10 to 40 times as sensitive as iodine for the detection of this carbohydrate. Approximately 1 part of starch in 900,000 parts of water can be detected.

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Determination of Ethers and Esters of Ethylene Glycol

A Modified Alkoxyl Analysis

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A modified alkoxyl method and apparatus have been devised by which the ethylene glycol content in esters and ethers may be determined quantitatively on a semimicro scale. Hot, constant-boiling hydriodic acid decomposes the ethylene glycol residue quantitatively into ethyl iodide and ethylene, which are collected and determined volumetrically in standard solutions of silver nitrate and bromine, respectively. The method has been applied to solvents, polyethylene ethers, plasticizers, and hydroxyethylcellulose, as well as to compounds containing halogen or sulfur substituents in place of oxygen upon the ethylene glycol residue.

SINCE, during recent years, derivatives of ethylene glycol have attained considerable technical importance as solvents, plasticizers, sizes, etc., there is frequently a need for an analytical method by which these compounds may be determined and identified.

To the author's knowledge, the only previously described method for the analysis of glycol ethers is that of Elkins (*6*) and Werner and Mitchell (*21*) which depends upon the chromic acid oxidation of the ether and titration of the excess dichromate. This method distinguishes readily between such compounds as monomethyl and ethyl ethers of ethylene glycol but is difficult to apply where the glycol ether is only a small part of a complex structure as in hydroxyethylcellulose or many glycol ether and ester plasticizers.

Methods for the analysis of methyl and ethyl ethers have been known since the work of Zeisel (*24*) who used constant-boiling hydriodic acid to split the ethers into the corresponding alkyl iodides.

$$ROR' + 2\,HI \longrightarrow RI + R'I + H_2O$$

The alkyl iodide then was distilled out and reacted with alcoholic silver nitrate to form silver iodide which was determined gravimetrically.

Pregl (*15*) adapted the Zeisel method to a micro scale and Vieböck and others (*19*) developed an iodometric method for determining the alkyl iodide. Further modifications in the apparatus were made by Clark (*2*), Elek (*5*), Vieböck (*19*), Samsel and McHard (*16*), and others (*12*).

None of these investigators reported any attempt to adapt the method to the analysis of 1,2-glycol ethers. Meisenheimer (*11*) found that ethylene glycol and hydriodic acid produced low and variable quantities of ethyl iodide. Grün and Bockisch (*8*) abandoned the idea of using the Zeisel method on glycols, after finding that ethylene and propylene glycols with constant-boiling hydriodic acid yielded about 50% of the alkyl iodides and gases identified as ethane and propane, respectively. The identification of these gases has now been shown to have been incorrect. Davis (*4*) applied the Zeisel analysis to phenoxyethanol and obtained low and variable results. Lawrie and others (*10*) obtained ethyl iodide by a Zeisel analysis of hydroxyethylcellulose, but did not report data to indicate what reproducibility was obtained. Small amounts of ethyl iodide have been reported from the decomposition of dioxane with hydriodic acid in a sealed tube at 100° (*13*).

Still other reports show that gaseous hydrogen iodide converts ethylene glycol to ethylene iodide (*17*) and concentrated hydriodic acid at 100° likewise decomposes diethylene glycol to ethylene iodide (*23*). Alkylene iodides have long been known to be unstable to heat and light (*1*, *14*, *18*) and this instability has been used for the preparation of pure olefins. Such decompositions into olefin and iodine are favored by the presence of iodide ions (*18*).

From the foregoing review, it appears probable that ethyl iodide and ethylene would be two of the products of the reaction of ethylene glycol derivatives and hot, concentrated hydriodic acid.

A preliminary macrodecomposition of polyethylene oxide with constant-boiling hydriodic acid and analysis of the gaseous products showed that the ethyl iodide and ethylene produced accounted quantitatively for the starting material. This finding shows why the various alkoxyl analyses fail to give quantitative and reproducible results with such compounds. If silver nitrate is used as the absorption medium for ethyl iodide, the ethylene escapes. If a bromine–acetic acid solution (*2*, *5*, *19*) is used, ethylene may react with the excess bromine, but it is not detected and does not interfere with the subsequent titration of the iodate ions from the oxidation of ethyl iodide. On the other hand, when the usual alkoxyl analysis is applied to determine methoxyl or ethoxyl groups in a compound containing ethylene glycol residues, the results are always too high for the alkoxyl content because of the ethyl iodide from the glycol residues.

The method presented here provides a means by which ethylene glycol residues or methoxyl and ethoxyl groups combined with ethylene glycol residues may be determined quantitatively.

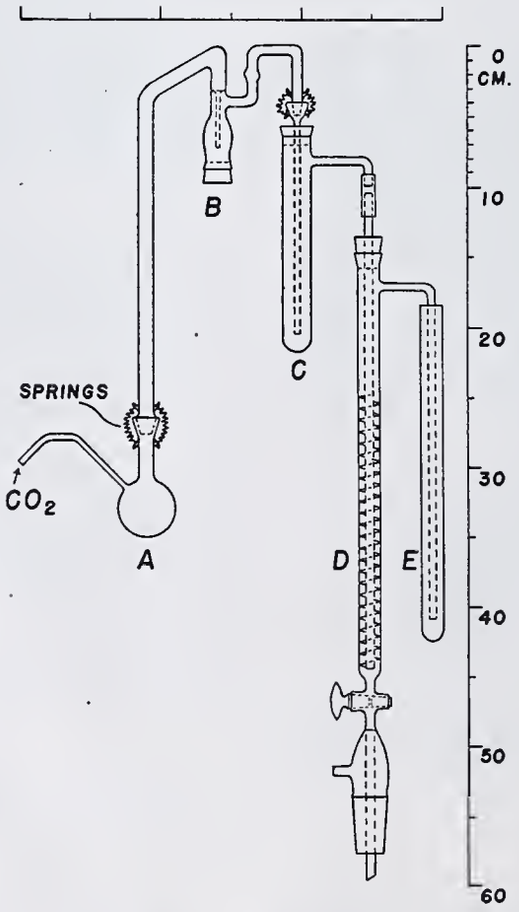

Figure 1. Apparatus

APPARATUS

An arrangement of apparatus for the analysis is shown in the scale diagram (Figure 1). It consists in part of the reaction flask, condenser, and first absorption tube of a Clark (*2*) alkoxyl apparatus. These are followed by an absorption tube, *D*, made from a section of a spiral from a Widmer distillation column and a standard-taper (24/40) gas inlet adapter.

Dimensions of the apparatus not readily determined from the diagram are as follows: carbon dioxide inlet capillary, 1-mm. inside diameter; flask *A*, 28-mm. diameter, 12/18 standard-taper joint; condenser, 9-mm. inside diameter; inlet to trap *B*, 2-

mm. inside diameter; inlet to trap *C*, 7/15 standard-taper joint, 2-mm. inside diameter tube; trap *C*, 14-mm. inside diameter; trap *D*, inner tube, 8-mm. outside diameter, 2-mm. opening at bottom of spiral; spiral, 1.75-mm. rod, 23 turns, 8.5-mm. rise per turn; outer tube, approximately 12.5-mm. inside diameter; side arm 7 cm. from top of inserted spiral, 3.5-mm. inside diameter, 2-mm. opening at bottom.

The stopcock is lubricated with Silicone grease. The absorption tubes may be conveniently suspended by a series of properly spaced sheet metal clips attached to a stick clamped at an angle of about 60°.

For the analysis of lowly substituted cellulose ethers a reaction flask of double the illustrated capacity is used. Although a minimum number of connections is desirable and an apparatus may be built with all ground-glass connections, no particular difficulty has been experienced with the rubber connections for tubes *C* and *D*, and they produce a desirable degree of flexibility.

REAGENTS

Hydriodic Acid. Constant-boiling hydriodic acid (specific gravity 1.70; boiling point 126–7° C.) is prepared by distilling reagent grade acid over red phosphorus in an atmosphere of carbon dioxide. Adequate precautions should be taken to avoid possible violent explosions of mixtures of air and phosphorus hydrides in the receiver. The acid should be distilled within a few days of the period of use or else stored after distillation in an atmosphere of carbon dioxide or nitrogen in dark-colored, sealed bottles. It should be free from sulfur compounds and from phosphine and hypophosphorous acid. Hypophosphorous acid, which is sometimes used as a stabilizer for hydriodic acid, may be destroyed by the addition of a few iodine crystals followed by distillation over red phosphorus. Phosphine in the distilled acid is indicated by unusual stability to atmospheric oxygen and by the blackening of silver nitrate paper held above the acid. This contamination is frequently present in acid distilled from phosphorus, but is readily removed by refluxing the hydriodic acid until the vapors no longer color silver nitrate paper gray or black. Hydriodic acid vapors stain paper brown or brownish black.

Silver Nitrate Solution. Silver nitrate (15 grams) is dissolved in 50 ml. of water and then added to 400 ml. of absolute ethanol. Several drops of concentrated nitric acid are added. This solution is standardized against 0.05 *N* ammonium thiocyanate by the Volhard method (*20*). It is very stable and shows very little change with use.

Bromine Solution. Bromine (1 ml.) is added to 300 ml. of c.p. glacial acetic acid saturated with dry potassium bromide (5 grams). Fifteen milliliters of this solution require about 40 ml. of 0.05 *N* sodium thiosulfate. This solution is stored in a dark bottle and kept in the dark. It is standardized at least once a day during use.

Alternatively, a methanolic solution of bromine and potassium bromide may be used (*9*). Absolute methanol (500 ml.) is saturated with dry potassium bromide (10 grams) and 1.8 ml. of bromine are added. Fifteen milliliters of this solution require between 40 and 50 ml. of 0.05 *N* sodium thiosulfate. This solution is stored as directed above. It is standardized against sodium thiosulfate with each analysis.

Other Reagents. The following required solutions need no special description:

Potassium iodide, 10% aqueous solution.
Sulfuric acid, 10% aqueous solution.
Sodium thiosulfate, 0.05 *N* standard solution.
Ammonium thiocyanate, 0.05 *N* standard solution.
Starch indicator, 1% aqueous solution.
Ferric ammonium sulfate indicator, saturated aqueous solution (filtered).

SAMPLE SIZE

The choice of sample size is important for accuracy and complete decomposition. Samples of very high ether content should weigh from 50 to 100 mg. and may require 1, 2, or more hours for complete decomposition. For samples containing less than 10% of glycol residue, 200 to 300 mg. of material and a reaction flask of twice the illustrated capacity are used. The amount of hydriodic acid is correspondingly increased.

PROCEDURE OF ANALYSIS

The apparatus is cleaned and dried. Trap *B* is filled with a suspension of a small amount of red phosphorus in enough water to cover the inlet tube. If the sample to be analyzed contains sulfur, the phosphorus is suspended in 5% aqueous cadmium sulfate solution instead of water. Ten milliliters of silver nitrate solution are pipetted into the first absorption tube, *C*, 15 ml. of bromine solution are pipetted into the spiral absorption tube, *D*, and 10 ml. of 10% potassium iodide solution are placed in the final tube, *E*. A weighed sample of glycol ether (0.05 to 0.12 gram) is placed in reaction flask *A*, together with a Hengar boiling granule and 10 ml. of hydriodic acid. When the sample has poor solubility in the acid or tends to sublime, a mixture of 1 ml. of phenol and 2 ml. of propionic anhydride is added to the reaction flask, as recommended by Elek (*5*) (*cf. 16*). The flask is connected to the apparatus, a slow stream of carbon dioxide (1 bubble per second) is passed through, and the flask is heated slowly with an oil bath to 140° to 145° C.

The flask is kept at this temperature a minimum of 40 minutes and frequently 1 to 2 hours are required for compounds with very high ether contents. Two indications of the completion of the decomposition are the absence of any cloudy reflux in the condenser above the reaction flask and the nearly complete clarification of the supernatant liquid in the silver nitrate trap. Five minutes before the completion of the reaction the silver nitrate trap is heated to 50° to 60° C. with a hot-water bath to drive out any dissolved olefin.

At the completion of the decomposition, tubes *D* and *C* are disconnected cautiously in that order. The carbon dioxide source then is disconnected and the heat removed from flask *A*. The spiral absorption tube, *D*, is then connected by its lower adapter to a 500-ml. iodine-titration flask containing 10 ml. of 10% potassium iodide solution and 150 ml. of water. The potassium iodide tube, *E*, is removed and the side arm rinsed into it. The bromine solution is allowed to run into the titration flask through the stopcock and the tube and spiral are rinsed with water. The contents of the potassium iodide tube are added to the titration flask, which is then stoppered and allowed to stand 5 minutes. Five milliliters of 10% sulfuric acid are added and the solution is titrated at once with 0.05 *N* sodium thiosulfate, using 2 ml. of starch indicator solution for the end point.

The contents of the silver nitrate trap are rinsed into a flask, diluted to 150 ml. with water, heated to boiling, cooled to room temperature, and titrated with 0.05 *N* ammonium thiocyanate, using 3 ml. of ferric ammonium sulfate solution as an indicator (*20*).

CALCULATIONS

After the titrations of the bromine and silver nitrate traps are subtracted from the corresponding blank titrations, the following calculations hold for ethylene glycol ethers:

$$\frac{\text{Difference in ml. of } Na_2S_2O_3 \times N \times 2.203}{\text{wt. of sample}} = \%\ C_2H_4O \text{ as } C_2H_4$$

$$\frac{\text{Difference in ml. of } NH_4SCN \times N \times 4.405}{\text{wt. of sample}} = \%\ C_2H_4O \text{ as } C_2H_5I$$

The C_2H_4O unit has been chosen as convenient for calculating the results of the analysis of compounds having greatly differing structures. Other groups, such as $—C_2H_4—$, $—OCH_2CH_2O—$, or $HOCH_2CH_2O—$, may be used by simply changing the factors in the equations and their use may appear more logical when dealing with particular types of derivatives.

CORRECTION FOR REAGENTS

Repeated blank determinations on the reagents in the apparatus showed that there was no change in the silver nitrate solution and there was an average loss in the bromine solution equivalent to 0.30 ml. of 0.05 *N* sodium thiosulfate. This correction was applied to all the determinations made using methanolic bromine–potassium bromide and bromine–acetic acid for the absorption of ethylene.

ANALYTICAL DATA

Tables I to IV contain the results of the analysis of various typical substances containing ethylene glycol residues.

THEORY OF PROCEDURE

When an ethylene glycol ether or ester is decomposed by constant-boiling hydriodic acid, the points of attachment of the ether and ester linkages are replaced by iodo and hydroxyl groups. Eventually all aliphatic hydroxyl groups are replaced by iodine or hydrogen, while phenolic hydroxyls and carboxyl groups are

unchanged. Reports in the literature (*17*, *23*) indicate that ethylene iodide can be isolated as an intermediate in the reaction of ethylene glycol or diethylene glycol with hydriodic acid and the formation of the final products, ethyl iodide and ethylene, is best explained by assuming the intermediate production of ethylene iodide.

$$ROCH_2CH_2OH + 3\,HI \longrightarrow RI + ICH_2CH_2I + 2\,H_2O \quad (1)$$

The exact course which the reaction takes preceding the formation of ethylene iodide probably varies with the number and type of substituents upon the glycol residue. One may reasonably assume that in many cases ethylene iodohydrin precedes ethylene iodide. In the case of polyethylene ethers, ethylene glycol, ethylene iodohydrin, and ethylene iodide may all be simple primary decomposition products.

As soon as ethylene iodide has been formed, several courses of further reaction are possible: the diiodide may decompose into iodine and an olefin, which escapes; part of the alkylene iodide may be reduced by hydriodic acid to alkyl iodide; part of the olefin formed by the first reaction may react with hydriodic acid to produce alkyl iodide.

Table I. Analysis of Ethylene Glycol Derivatives

Substance	Formula	Ethylene Trap	Per Cent —C_2H_4O— Found: As RI	Found: As C_2H_4	Found: Total	Calculated
Ethylene glycol di-O-(pentaacetyl-*d*-gluconate)[a]	$C_2H_4O_2[C_6H_6O_6(COCH_3)_6]_2$	MeOH–Br_2–KBr	4.45	0.91	5.36	5.25
		MeOH–Br_2–KBr	3.72	1.52	5.24	
		MeOH–Br_2–KBr	3.45	2.01	5.46	
2-Phenoxyethanol	$C_6H_5OCH_2CH_2OH$	MeOH–Br_2–KBr	21.83	10.16	31.99	31.88
		MeOH–Br_2–KBr	27.29	4.65	31.94	
		MeOH–Br_2–KBr	22.84	9.01	31.85	
		Br_2–CCl_4	23.13	8.79	31.92	
		Br_2–CCl_4	21.99	11.09	33.08	
		Br_2–CCl_4	20.12	12.61	32.73	
		Br_2–CH_3COOH	24.97	6.35	31.32	
		Br_2–CH_3COOH	28.42	3.39	31.81	
		Br_2–CH_3COOH	17.82	14.17	31.99	
Diethylene glycol dicresyl ether	$C_7H_7OC_2H_4OC_2H_4OC_7H_7$	MeOH–Br_2–KBr	19.68	11.28	30.96	30.76
		MeOH–Br_2–KBr	13.89	16.99	30.88	
		MeOH–Br_2–KBr	20.87	9.73	30.60	
		Br_2–CH_3COOH	14.80	16.11	30.91	
Ethylene glycol	$HOCH_2CH_2OH$	MeOH–Br_2–KBr	57.36	14.37	71.73	70.97
		MeOH–Br_2–KBr	42.07	29.56	71.63	
Diethylene glycol	$HOC_2H_4OC_2H_4OH$	MeOH–Br_2–KBr	69.68	13.22	82.90	83.05
		MeOH–Br_2–KBr	59.69	22.58	82.27	
Polyethylene oxide[b]	$HO(C_2H_4O)_{91}H$	MeOH–Br_2–KBr	82.48	15.57	98.05	99.59
		MeOH–Br_2–KBr	85.15	12.45	97.60	
		MeOH–Br_2–KBr	81.88	15.39	97.27	
		Br_2–CCl_4	60.11	42.18	102.3	
Dioxane	$(C_2H_4O)_2$	MeOH–Br_2–KBr	90.40	9.34	99.74	100.00
		MeOH–Br_2–KBr	86.90	11.54	98.44	
2-Methoxyethanol[c]	$CH_3OCH_2CH_2OH$	MeOH–Br_2–KBr	105.8	9.47	115.3	115.8
		MeOH–Br_2–KBr	102.6	11.21	113.8	

[a] For preparation see (*22*). [b] Carbowax-4000 of Carbide and Carbon Chemicals Corp.
[c] Methoxyl content calculated as —C_2H_4O—.

Table II. Analysis of Commercial Plasticizers

Substance	Formula	Ethylene Trap	Per Cent —C_2H_4O— Found: As RI	Found: As C_2H_4	Found: Total	Calculated[a]
Methoxyethyl oleate[b]	$CH_3OC_2H_4OOC_{18}H_{33}$	MeOH–Br_2–KBr	21.64	2.07	23.71	25.88
		MeOH–Br_2–KBr	20.32	3.03	23.35	
		MeOH–Br_2–KBr	21.96	1.89	23.85	
Triethylene glycol di-2-ethylhexoate[c]	$C_6H_{12}O_2(C_7H_{15}COO)_2$	MeOH–Br_2–KBr	23.48	7.79	31.27	32.82
		MeOH–Br_2–KBr	24.94	6.36	31.30	
		MeOH–Br_2–KBr	19.23	11.75	30.98	
		MeOH–Br_2–KBr	22.25	9.37	31.62	
		Br_2–CCl_4	19.74	11.71	31.45	
β-Ethoxyethyl-*o*-benzoyl benzoate	$C_6H_5COC_6H_4COOC_2H_4OC_2H_5$	MeOH–Br_2–KBr	24.55	3.75	28.30	29.54
		MeOH–Br_2–KBr	23.38	4.66	28.04	
Butoxyethylphthalate	$C_6H_4(COOC_2H_4OC_4H_9)_2$	MeOH–Br_2–KBr	18.13	6.94	25.07	24.05
		MeOH–Br_2–KBr	18.20	5.38	23.58	

[a] Methoxyl and ethoxyl groups calculated as —C_2H_4O—.
[b] Kapsol as received from Ohio Apex, Inc.
[c] Flexol 3GO of Carbide and Carbon Chemicals Corp.

$$ICH_2CH_2I \longrightarrow CH_2{=}CH_2 + I_2 \quad (2)$$

$$ICH_2CH_2I + HI \longrightarrow CH_3CH_2I + I_2 \quad (3)$$

$$CH_2{=}CH_2 + HI \longrightarrow CH_3CH_2I \quad (4)$$

In any case, the two final products are ethyl iodide and ethylene and the sum of these products is equivalent to the glycol ether groups in the substance. There may be present, of course, volatile alkyl halides from other sources. No paraffin hydrocarbons are produced.

The following equation is a summation of Equations 1 to 4, in which x is a variable number less than 1:

$$ROCH_2CH_2OH + (3 + x)\,HI \longrightarrow RI + (x)\,CH_3CH_2I + (1 - x)\,CH_2{=}CH_2 + I_2 + 2\,H_2O \quad (5)$$

The ratio of ethyl iodide and ethylene obtained varies with several reaction conditions, as is pointed out in the discussion on application of the procedure. No exception to the above course of reaction has been found among the wide variety of structures represented in the tables. Other 1,2-glycols and their derivatives are decomposed by hydriodic acid in a similar way forming alkyl iodides and olefins.

DEVELOPMENT AND APPLICATION OF METHOD

In developing an absorption system a single silver nitrate tra was found to be sufficient to absorb all alkyl iodide vapors Simple traps of bromine solution would not absorb all of th ethylene, even when several were used in series. Therefore, th spiral tube was devised to provide a longer contact of the gase with the bromine solution. The standard-taper adapter wa added to this tube to permit the transfer of the bromine solutio to a titration flask without loss. It was found advisable to plac the side arm well above the upper end of the spiral or to enlarg the upper part of the tube into a bulb in order to provide roon for the contents of the tube if the flow of gas through the systen became too rapid. Rushes of gas frequently occurred just a the reaction was brought to boiling. Caution must therefore b taken in bringing the mixture to a boil, even though good boilin stones are provided.

A final simple trap containing potassium iodide solution wa added to the system to collect any bromine swept out by th flow of carbon dioxide. Practically no bromine was found to es cape from methanolic bromine–potassium bromide solution while bromine equivalent to 0.75 ml. of 0.05 *N* sodium thiosulfat was often obtained in 40 minutes from bromine–acetic acid solu tion. The volatility of the bromine was reduced by saturatin the solution with potassium bromide.

A solution of bromine in carbon tetrachloride was unsatisfactory for the absorption of ethylene because of the volatility of the solution. The resulting losses produced values for the percentage of glycol residues which were too high (Table I). Bromine in methanol saturated with potassium bromide was used in the majority of determinations reported. This solution was very easily handled without bromine losses, but the titer dropped rapidly during storage and use and it therefore required frequent standardization. Occasionally solutions were obtained which were very unstable and gave high blank determinations. These blanks may be reduced to small and reproducible values by storing new solutions for at least 10 days before use (*2A*). An acetic acid solution of bromine had the best stability to storage and use and was readily handled without losses.

Because a single absorption system would greatly simplify the determination of ethylene glycol derivatives, several attempts were made to achieve this end. In one experiment the vapors from the reaction flask, *A*, were passed through a second heated flask of hydriodic acid. No marked increase in the yield of ethyl iodide was obtained.

Another approach to a simplified absorption system is the use of a single standard bromine solution, which reacts with both the alkyl iodide and olefin from glycol derivatives but not in an equivalent manner.

$$RI + Br_2 \longrightarrow RBr + IBr$$

$$IBr + 3\,H_2O + 2\,Br_2 \longrightarrow HIO_3 + 5\,HBr$$

$$CH_2{=}CH_2 + Br_2 \longrightarrow CH_2BrCH_2Br$$

The reactions represented by the first two equations are the basis of the Vieböck absorption system for alkyl iodides (*2*, *19*). If a standardized selective reducing agent could be found which would react with the excess bromine and not with the iodate ions, this single solution system could be used for the determination of glycol ethers. The alkyl iodide would be determined by titration of the iodate ions and the olefin by the difference between the total bromine at the beginning and the sum of the excess bromine and that used to react with alkyl iodide. Standard formic acid reacts selectively but not stoichiometrically with the excess bromine and attempts to use it for this purpose were therefore unsuccessful.

Except for various adjustments in the adopted procedure to accommodate samples of varying ether content and solubility, as noted earlier, most derivatives of ethylene glycol were analyzed without difficulty.

Several factors were found to affect the ratio of alkyl iodide and olefin obtained. Factors which promoted the formation of high percentages of alkyl iodide were (1) freshly distilled hydriodic acid, (2) small sample size, and (3) low temperatures during the first part of the reaction. The values obtained in the analysis of ethylene glycol di-O-(pentaacctyl-*d*-gluconate) (Table I) illustrate the effect of aging hydriodic acid, which for succeeding samples was 1, 2, and 6 days old, respectively. The ratio of ethyl iodide to ethylene in general had no bearing on the total result, except that, if the amount of ethylene became too great and was evolved too rapidly, the absorption tube failed to take it up completely. No combination of conditions was found which yielded 100% alkyl iodide in a period of time suitable for an analytical method.

Chlorine-containing compounds required no changes in the procedure, but sulfur-containing compounds required the use of cadmium sulfate in the phosphorus scrubbing trap in order to collect the hydrogen sulfide which was evolved.

Thiodiglycol (Table III) produced only a 50% yield of ethylene and ethyl iodide and a crystalline sublimate. The sublimate was identified as 1,4-dithiane (melting point 110°) rather than β,β'-diiodoethyl-sulfide, which is reported to form very readily with thiodiglycol and hydriodic acid (*7*) 4,7-Dithiasebacic acid was successfully analyzed by using a solvent mixture of phenol and propionic anhydride in the flask and heating the reaction mixture at 110° for 2 hours before going higher.

In the analysis of butoxyethyl phthalate (Table II) much too high results were obtained unless the carbon dioxide flow was slowed down and the oil bath kept below a maximum of 140°. For the satisfactory separation of ethyl and methyl iodides from propyl and butyl iodides, a water-cooled condenser as used by Elek (*5*) and others would be required.

1,2-Propylene glycol ethers were found to follow the same course of reaction as ethylene glycol ethers, forming propylene and isopropyl iodide. The

Table III. Analysis of Miscellaneous Substances

Substance	Formula	Per Cent —C_2H_4O— Found: As RI	As olefin	Total	Calculated[a]
β,β'-Dichloroethyl ether	$(ClCH_2CH_2)_2O$	47.73	12.87	60.60	61.61
		50.89	10.30	61.19	
4,7-Dithiasebacic acid	$CH_2S{-}C_2H_4COOH$	13.36	5.02	18.38	18.48
	$CH_2S{-}C_2H_4COOH$	12.54	6.15	18.69	
Thiodiglycol	$(HOCH_2CH_2)_2S$	26.29	9.41	35.70	72.10
		13.06	24.09	37.15	
		31.49	7.88	39.37	
Dipropylene glycol	$(CH_3CHOHCH_2)_2O$	45.34	12.65	57.99	58.67
		44.33	13.14	57.47	

[a] Alkylene groups in samples calculated to theoretical yield of —C_2H_4O—.

Table IV. Typical Analyses of Hydroxyethylcellulose

Sample	Moles of Ethylene Oxide Used per Glucose Unit	Per Cent —C_2H_4O— Found: As C_2H_5I	As C_2H_4	Total	Hydroxyethyl Groups per Glucose Unit From modified alkoxyl method	From saponification of triacetate	Solubility
A	0.25	3.06	0.54	3.60	0.17	..	Soluble in 7% NaOH
		3.14	0.66	3.80	0.17	..	Soluble in 7% NaOH
B	0.50	4.52	2.06	6.58	0.26	..	Soluble in 7% NaOH
		5.18	1.16	6.34	0.25	..	Soluble in 7% NaOH
C	0.75	8.45	2.08	10.53	0.43	..	Swollen by cold H_2O
		7.04	3.18	10.22	0.42	..	
D	1.50	14.77	2.08	16.85	0.75	..	Swollen by many solvents; not swollen by acetone
		13.89	2.20	16.09	0.71	..	
E[a]	4.05	28.25	5.60	33.85	1.88	..	Soluble in H_2O, AcOH, and C_5H_5N
		19.02	13.72	32.74	1.79	..	
F[a]	10.0	30.96	21.66	52.62	4.09	..	Soluble in H_2O and aq. EtOH; insoluble in acetone
		35.64	17.16	52.80	4.12	..	
Ceglin D[b]	...	3.34	1.32	4.66	0.18	..	Soluble in 7% NaOH
		3.49	1.23	4.72	0.18	..	
Ceglin H[b]	...	3.12	3.07	6.19	0.24	..	Soluble in 7% NaOH
		3.44	2.12	5.56	0.22	..	
Cellosize WS-100[a,c]	...	20.32	7.82	28.14	1.44	1.45	Soluble in H_2O and nearly soluble in AcOH or C_5H_5N
		22.65	5.61	28.26	1.45	1.49	
		23.15	4.89	28.04	1.44	..	

[a] Analytical values corrected for ash content.
[b] Product of Sylvania Industrial Corp.
[c] Product of Carbide and Carbon Chemical Corp.

latter was distilled from the reaction flask with considerable difficulty and it was necessary to heat the condenser and the phosphorus trap and keep a very high reflux in order to drive it over.

DISCUSSION

The data show that the precision and accuracy of the method are satisfactory for the analysis of a wide variety of glycol derivatives. The columns in the tables showing the percentages of alkyl iodide found are the values which would have been obtained if the substances had been analyzed by the Zeisel or Vieböck methods. In general, these values are low and variable. The yields of alkyl iodide in duplicate analyses are often of a similar magnitude because consecutive analyses were performed under similar conditions. When the percentage of glycol units determined as ethylene is added to the percentage as alkyl iodide, the total checks well with the calculated values. The duplicate values found for a number of unpurified plasticizer samples (Table II) agree well among themselves but are all lower than the calculated values. The probable presence of impurities accounts for these results.

Substances or structures which would interfere with the analysis are any volatile alkyl iodide from an ether, ester, sulfide, etc., or structures which might produce 1,2-diiodides and eventually gaseous olefins but from which relatively nonvolatile monoiodides would result. Methyl, ethyl, and possibly isopropyl iodide, if they are known to be potentially present, may be easily taken into account by calculating the over-all results as glycol residue, as was done in the case of methoxy- and ethoxyethanol derivatives in this work (Tables I and II). Acetone is an interfering substance because it reacts readily with bromine. If it is used for drying purposes, the apparatus must be carefully freed of it before the analysis is performed.

Methoxyl and methyl ester groups may be determined in the presence of glycol residues by a separate analysis according to the recent modification of the Willstätter method by Cooke and Hibbert (*3*). Of course, methyl and ethyl ester groups, as well as other ester groups, may also be determined separately by saponification.

The correct degree of substitution of various hydroxyethylcellulose derivatives is presented here for the first time (Table IV). Most previous estimations have been based upon the amount of etherifying agent used or upon the gain in weight of the product. Obviously the method of analysis described here does not determine whether the hydroxyethyl ether groups are present as individual units or are built up in polyethylene oxide units of varying sizes. It seems more than probable that the latter is increasingly the case as the substitution is increased and it must be so in the case of sample F (Table IV) in which 4.1 glycol residues are distributed over 3 cellulose hydroxyls.

An independent check on the hydroxyethyl content in cellulose derivatives was obtained in the case of Cellosize WS-100 by preparing the triacetate with pyridine and acetic anhydride and calculating the number of glycol residues necessary to produce the observed difference in acetyl value between this sample and cellulose triacetate. Such a method is applicable to the more highly substituted hydroxyethylcellulose derivatives.

Since a direct analysis of hydroxyethylcellulose has not previously been realized, a few comments on the relation of substitution to the method of preparation and solubility of these derivatives are in order. The data in Table IV show that the efficiency of the reaction between ethylene oxide and alkali cellulose (18% sodium hydroxide; 33% cellulose) varied from as high as 68% down to 40% as the portions of ethylene oxide were increased from 0.25 to 1.0 mole. Larger amounts of ethylene oxide were added in successive 1-mole portions. Considerably higher efficiencies were obtained by adding the ethylene oxide in smaller portions.

Certain generalities concerning the solubility of these derivatives in relation to substitution are not clear from the number of analyses presented. Above 0.35 mole hydroxyethyl groups pe glucose unit (g. u.), the derivatives become increasingly sensitiv to cold water and eventually dissolve at substitutions betwee 1.0 and 1.2 moles per g. u. Up to 0.5 mole per g. u. they may b handled in hot water. Above 0.75 mole per g. u. they are con siderably swollen by alcohols and acetic acid but not by acetone Solubility in powerful organic solvents is attained at about 1. moles per g. u. and eventually solubility in common solvents i obtained. These solubility rules are based on derivatives havin degrees of polymerization between 500 and 800 anhydroglucos units. In contrast with other cellulose ethers, hydroxyethyl cellulose ethers show increasing solubility in all media with in creasing substitution. In other words, once solubility in a give solvent is reached, it is not lost by increasing the hydroxyethy substitution.

In conclusion, in addition to the analysis of simple ethylen glycol derivatives as described, the method may be used for th determination of glycol derivatives in mixtures, such as aqueou solutions or plasticized polymeric materials. It is believed tha the method may be applied by proper adaptation to many othe types of compounds containing chains of two methylene group linked to noncarbon functional groups and should prove usefu for the identification and proof of structure of such compounds.

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Precision Semimicromethod for Gas Analysis

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An apparatus is described which is capable of complete analysis of small samples of carbon dioxide, carbon monoxide, methane, hydrogen, nitrogen, and/or oxygen. The operations of analysis are simple and reliable, and the danger of loss of a valuable sample is reduced to a minimum. With a sample of volume approximating 1 cc. at N.T.P. an accuracy of a few tenths of a per cent of the total gas sample may be anticipated; but an accuracy of about 1% is maintained with samples as small as 0.1 cc. N.T.P. The conditions for a satisfactory fractional combustion on a platinum catalyst of hydrogen and carbon monoxide in the presence of methane and excess oxygen are defined.

IN THE course of an investigation of the gases in meteorites a sensitive method for the analysis of low-pressure gases has been developed, which provides data of high accuracy with very small gas samples, and in which the danger of accidental loss of the sample is reduced to a minimum.

Figure 1 illustrates the apparatus, in which all the measurements are made at diminished pressures.

Only solid adsorbents are employed, and Apiezon stopcock grease is used throughout. The 1000-cc. reservoir, *A*, containing purified mercury, is linked with the 500-cc. chamber of the Töpler pump, *B*. The rise and fall of the mercury in *B* is controlled through stopcock 1, whose two leads give access to an aspirator line and to the atmosphere, respectively. The buret, *C*, is made from 12-mm. Pyrex tubing, and is graduated from 1 to 10 cc. in steps of 1 cc. The buret bulb has an additional capacity of 10 cc., permitting the accommodation of particularly large gas samples. The barometer, *D*, is made from the same piece of tubing as *C*, and is provided with the overflow attachment, *E* (*2*). The difference of the height of the mercury columns in *C* and *D*, which represents the gas pressure in *C*, may be read accurately from a graduated mirror scale set behind the tubes. A calibrated thermometer set close to *C* indicates the gas temperature.

The drying tube, *F*, contains a small amount of Anhydrone, trap *G* permits convenient separation of any condensable material in the gas sample, and tube *H* contains Asearite, for the absorption of carbon dioxide and other acid gases. The combustion catalyst tube, *J*, consists of a short section of quartz capillary tubing, connected to the rest of the line through ground quartz to Pyrex joints. The capillary contains a 5-cm. length of No. 22 platinum wire, and is heated by a small closely fitting furnace with Alundum core, Nichrome winding, and asbestos lagging. The presence in each branch of the adsorption train of a small spiral spring made of 4-mm. (outside diameter) Pyrex tubing (not shown in the diagram) prevents any strains from developing in the line.

Stopcock 5 gives access to a series of bulbs containing various pure gases and determinate gas mixtures, while stopcock 6 leads to the line from which the gas sample is to be collected, and thence to the evacuating pumps. For cases where a small gas sample is to be collected from a very large volume it has proved advantageous to precede stopcock 6 with a small volume and a mercury diffusion pump in that order. The diffusion pump rapidly concentrates the gas sample in the small volume, from which it may be readily conveyed to the buret by the Töpler pump.

In determining the graduations of the buret, the 10-cc. mark was determined directly, by weighing the mercury delivered through a stopcock temporarily attached to the bottom of the buret before the latter was sealed in place. The other points are then most simply determined after the buret has been permanently mounted—e.g., by compressing a sample of gas to the 10-cc. mark, reading the pressure, and then slowly compressing it until the new pressure, corrected for any small fluctuations in temperature, is just 10/9 the former value. The new position of the mercury meniscus in the buret now corresponds to 9 cc., and may be marked as such. The other buret graduations are located in similar fashion. Nitrogen is used as the calibration gas and, by occasionally discarding part of the gas sample, the pressures are kept below 200 mm., so that no errors due to gas imperfection can be introduced.

Calibration by this method is advantageous because the buret is calibrated after it has been permanently set in place; and is calibrated in the same way, and with the same precision, as in later use. Furthermore, it is somewhat simpler to secure markings corresponding exactly to unit numbers of cubic centimeters by this method than by any other method.

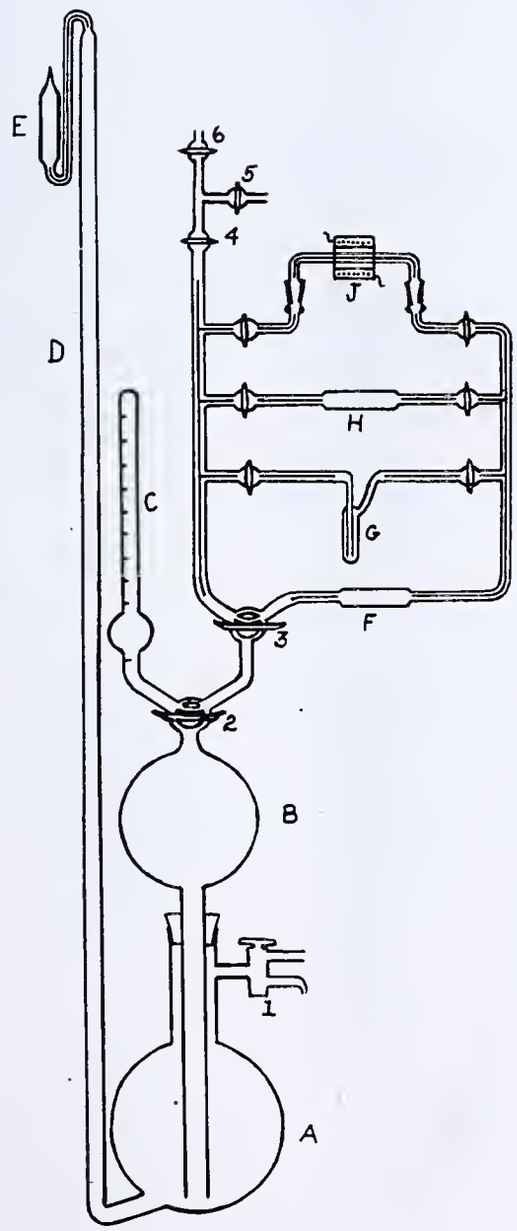

Figure 1. Analytical Train

OPERATIONS OF ANALYSIS

The dry gas sample is collected in the buret by repeated strokes of the Töpler pump, and the mercury is run up into the buret until the gas is compressed to that mark which most nearly corresponds to a sample pressure of 150 mm. The pressure is then determined with an accuracy of 0.1 mm., the temperature is read to the nearest 0.1° C., and, since the probable accuracy of the volume calibration is 0.001 cc., it becomes possible to compute the gas volume under standard conditions with a probable accuracy of 0.1%.

This calculation is predicated upon the behavior of the sample as a perfect gas, a valid assumption in this instance, since it has been shown (*1*) that under pressures of 150 mm. even a gas as imperfect as carbon dioxide behaves relatively ideally. Furthermore, any volume change produced by mixing is of negligible magnitude. Measurement at diminished pressure, as practiced in this mode of analysis, therefore not only serves to increase the effective gas volume to a readily determinable magnitude, but also obviates any aberrations due to imperfection of the gases involved.

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Consider now the operations involved in the analysis of a sample containing carbon dioxide, carbon monoxide, methane, hydrogen, oxygen, and nitrogen. After measurement of the total original volume, the sample must be circulated over the Ascarite to absorb the carbon dioxide. To perform this operation the analytical train is first thoroughly evacuated, and then shut off from the evacuating pumps. The gas is withdrawn from the buret with the Töpler pump (ordinarily two or three strokes are required to empty the buret completely), and passed into the absorption train through the left branch of the three-way stopcock, 3, the mercury rising as far as the base of stopcock 3. To complete the circulatory cycle, the bulk of the gas is collected through the right branch of stopcock 3, by turning the stopcock to the appropriate position and then lowering the mercury in the pump. To ensure perfectly complete absorption this cycle may be repeated two or three times. By circulating the gas over the absorbent in this fashion it is possible to avoid any danger of the dead gas diffusion blocking occasionally encountered in the Orsat apparatus. Finally, the residual gas sample is completely collected through the right branch of stopcock 3 with three or four strokes of the Töpler pump, and conveyed back into the buret where its temperature, pressure, and volume are again measured. Recovery of the residual sample is more than 99.9% complete after four strokes of the Töpler pump. By invariably collecting the residual gas through the right branch of cock 3 any water liberated by the Ascarite (or, later, in the combustion of hydrogenous material) is absorbed by the Anhydrone, and the gas reaching the buret is always in a state of uniform dryness.

To provide a small excess of oxygen for the impending combustion analysis, a suitable quantity of this gas is drawn from its storage bulb with the Töpler pump, and added to the sample in the buret, whereupon the new volume is measured. The difference between this volume and that of the previous measurement represents the volume of oxygen added. The diluted gas sample is then repeatedly circulated over the combustion catalyst, which is maintained at 475° C., and the residual gas is collected and remeasured.

The further operations of the analysis are carried through by the same general procedures as indicated above, and the complete analytical scheme is shown below in outline form. The volume, pressure, and temperature are measured after each of the following operations:

1. Complete collection of original sample
2. Circulation over Ascarite to remove carbon dioxide
3. Addition of oxygen in small excess, for combustion
4. Circulation over platinum catalyst at 450° C. to burn hydrogen and carbon monoxide
5. Circulation over Ascarite to remove carbon dioxide from carbon monoxide combustion
6. Circulation over platinum catalyst at 950° C. to burn methane
7. Circulation over Ascarite to remove carbon dioxide from methane combustion
8. Addition of hydrogen in small excess, for combustion of excess oxygen
9. Circulation over hot platinum catalyst to burn excess oxygen

All the readings of temperature, pressure, and volume collected in the above scheme are converted to the corresponding volumes at normal temperature and pressure. If the term v_x is made to refer to the N.T.P. volume after the x'th operation above, then the analytical results are given in the following set of formulas. It will be observed that, with the exception of oxygen, all the components are determined from relatively simple, direct formulas, and a relatively high order of accuracy may be obtained. The oxygen content is calculated indirectly and is, therefore, subject to cumulative errors. However, even in this unfavorable case, a probable accuracy of 0.5% may still be obtained.

$$\frac{\%CO_2}{100} = \frac{\text{volume of } CO_2}{\text{total volume}} = \frac{v_1 - v_2}{v_1}$$

$$\frac{\%CO}{100} = \frac{\text{volume of CO}}{\text{total volume}} = \frac{\text{volume of } CO_2 \text{ after 475° C. combustion}}{\text{total volume}}$$

$$= \frac{v_4 - v_5}{v_1}$$

$$\frac{\%H_2}{100} = \frac{\text{volume of } H_2}{\text{total volume}} = \frac{\text{2/3 (volume of contraction due to } H_2 \text{ combustion)}}{\text{total volume}}$$

$$= \frac{\text{2/3 (total 475° C. volume of contraction − contraction due to CO)}}{\text{total volume}}$$

$$= \frac{2/3\,[(v_3 - v_4) - 1/2\,(v_4 - v_5)]}{v_1} = \frac{2v_3 - 3v_4 + v_5}{3v_1} =$$

$$\frac{CO_2 \text{ volume after 850° C. combustion}}{\text{total volume}} = \frac{v_6 -}{v_1}$$

$$\frac{\%CH_4}{100} = \frac{\text{volume of } CH_4}{\text{total volume}}$$

$$= \frac{\text{1/2 (volume of contraction due to 850° C. combustion)}}{\text{total volume}} =$$

$$\frac{v_5 -}{2v_1}$$

$$\frac{\%N_2}{100} = \frac{\text{volume of } N_2}{\text{total volume}} = \frac{\text{(total } N_2 \text{ and } O_2 \text{ residue after 3i Ascarite passage − residual oxyge}}{\text{total volume}}$$

$$= \frac{v_7 - 1/3\,(v_8 - v_9)}{v_1} = \frac{3v_7 - v_8 + v_9}{3v_1}$$

$$\frac{\%O_2}{100} = \frac{\text{volume of } O_2}{\text{total volume}} = \frac{\text{(}O_2 \text{ theoretically used − } O_2 \text{ apparently used)}}{\text{total volume}}$$

$$= \frac{\text{(}O_2 \text{ equivalent of } H_2, \text{ CO, and } CH_4 - O_2 \text{ apparently used)}}{\text{total volume}}$$

$$= \frac{1/2\,v_{H_2} + 1/2\,v_{CO} + 2\,v_{CH_4} - (O_2 \text{ put in} - O_2 \text{ residue})}{\text{total volume}}$$

$$= \frac{\dfrac{2v_3 - 3v_4 + v_5}{6} + \dfrac{v_4 - v_5}{2} + 2\,(v_6 - v_7) - (v_3 - v_2) + 1/3\,(v_8 - v_9)}{v_1}$$

$$= \frac{3v_2 - 2v_3 - v_5 + 6v_6 - 6v_7 + v_8 - v_9}{3v_1}$$

FRACTIONAL COMBUSTION

The fractional combustion on a platinum catalyst of hydrog and carbon monoxide in the presence of methane and exce oxygen is the vital step in this procedure, for the validity which sufficient evidence is not to be found in the literatur Although fractional combustion on oxide catalysts, especial cupric oxide, has long been known and practiced, it does n appear to be expedient if a determination of oxygen is in prospec since these "catalysts" may themselves furnish a portion of tl oxygen used in the combustion. Therefore, the possibility using a noble metal catalyst in fractional combustion process has been studied.

A somewhat analogous case has been investigated by Richar (*5*), using a heated palladium tube. In the course of his r search he noted that if hydrogen were mixed with methan combustion of methane occurred at temperatures far too low burn it when it was unmixed with hydrogen. This phenomen was attributed to the strong heating of the catalyst by the cor bustion of the relatively large quantities of hydrogen presen and though this difficulty has been rather generally noted

ractional combustion work, it is not to be anticipated in the resent instant in view of the low pressures of the gases involved.

Because of the cost, fragility, and tendency of a palladium tube atalyst to develop leaks, preliminary tests were made to discover whether an entirely enclosed platinum catalyst could be sed for the fractional combustion.

In the first trial 1 gram of platinized asbestos, previously eated to redness in air, was packed into the quartz tube. It as then found that when measured small quantities of oxygen ere circulated over the catalyst, while the latter was heated to right redness, 1 to 2% of the sample was lost. A second passage f the same gas sample over the catalyst resulted in a further loss f 1 to 2%, so that the loss cannot be attributed to a combustible npurity in the oxygen, which, moreover, had already been reated with hot platinized asbestos during its purification. Consequently, the asbestos was discarded in favor of a small roll of latinum foil. The oxygen loss was then reduced to 0.5%.

The combustion catalyst tube was then remodeled along lines riginally suggested by Hempel (*4*), and the combustion catalyst onsisted of a 5-cm. length of No. 22 platinum wire inserted in ne 2-mm. bore of the quartz combustion capillary tube. With nis catalyst renewed tests with small measured oxygen samples evealed a loss of only 0.1% when the sample was circulated over ne catalyst at 475° C., and a loss of 0.2% when the circulation as performed at 850° C. These losses were accurately reproucible, and since they were close to the limiting experimental rror, no further attempt to reduce them has been made.

The explanation of these oxygen losses, while it may lie in a race of copper in the platinum, probably resides in physical dsorption or solution of oxygen by the platinum. Such abrption has been reported by Sieverts (*6*) as increasing with sing temperature, in line with the present observations. Furnermore, the recovery of such dissolved gas would be extremely ow (*7*), in line with the author's experience that, after the assage of oxygen over the catalyst, prolonged evacuation is reuired before the combustion tube can be pumped flat.

A very small, though unmistakable, loss of oxygen (circa 0.05%) hen a measured sample of this gas was circulated through other arts of the line is almost certainly attributable to reaction of the xygen with the stopcock grease, especially since a 100% reovery was always secured when measured quantities of other ases were circulated through the line. The error in the case of xygen is negligible in any event.

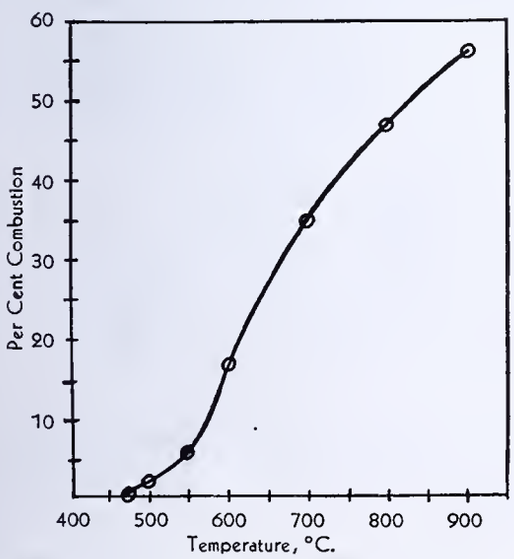

Figure 2. Combustion of Methane on a Platinum Catalyst

With the final form of catalyst tube the extent of combustion f methane-oxygen mixtures, as a function of catalyst temperaure, was studied. The results shown in Figure 2 represent the er cent combustion during a single slow passage through the catalyst tube of a uniform methane-oxygen mixture containing excess oxygen. It is apparent that at 475° C. only about 0.1% of the methane is burned on passage over the catalyst, yet at this temperature hydrogen-oxygen and carbon monoxide–oxygen mixtures were found to burn rapidly and completely on the catalyst. Consequently, the preliminary data collected with these binary mixtures strongly indicate the possibility of a successful low-pressure fractional combustion on a platinum catalyst. That such fractionation does occur satisfactorily even for mixtures of all the combustible components is proved by the outcome of control analyses.

Table I. Tests of Synthetic Mixtures

Analysis No.	Total Volume of Sample *Cc. N.T.P*		H_2 %	CO %	CH_4 %
1	1.104	Given	55.7	28.15	16.15
		Found	55.7	28.3	16.0
2	1.031	Given	58.3	31.1	10.6
		Found	58.0	31.4	10.6
3	0.926	Given	64.45	30.1	5.5
		Found	64.3	30.3	5.4
4	1.237	Given	79.3	17.3	3.5
		Found	79.1	17.5	3.45
5	1.258	Given	52.9	43.3	3.8
		Found	52.9	43.3	3.9

CONTROL ANALYSES

In preparation for the control trials, several samples of pure gases were prepared.

Hydrogen and oxygen were generated in an all-glass cell, by electrolysis of 10% sulfuric acid between platinized platinum electrodes, and purified in identical trains by successive passage over fused potassium hydroxide, hot platinized asbestos, fused potassium hydroxide, and phosphorus pentoxide. The hydrogen so prepared assayed 100% pure when burned with excess oxygen; and the oxygen, when freshly prepared, assayed 100% pure when burned with excess hydrogen.

Despite the original high purity of the oxygen, it was found to contain small but appreciable quantities of volatile, partially oxygenated organic compounds after standing for some months in the storage bulb. The source of this material probably lies in the vaseline-rubber (Ramsay) grease originally used to lubricate the bulbs' stopcocks. Although it has been known for some time that oxygen attacks such grease (*3*), the formation of volatile reaction products does not appear to have been previously noted. Considerably better though not perfect preservation was found when the Ramsay grease was replaced with Apiezon M. Analytical aberrations due to the progressive contamination of the oxygen may be avoided by always drawing the oxygen into the analytical train through a liquid air trap packed with glass wool; or by preparing a fresh supply of oxygen about once a month.

Carbon monoxide was generated by the action of hot 85% phosphoric acid on 85% formic acid (*8*). The gas was purified by passage through a sodium hydroxide scrubber and over fused potassium hydroxide and phosphorus pentoxide. The final purity, as assayed by combustion with excess oxygen, was 99.9%. The trace of impurity may have been due to slightly incomplete removal of dissolved air in the formic acid.

Methane was prepared by the cautious pyrolysis of an equivalent mixture of sodium acetate and sodium hydroxide. This crude gas was purified by successive passage through a dust trap, a dry ice trap (for the removal of water vapor), a packed dry ice trap containing a good grade of coconut charcoal pre-

viously outgassed at 500° C. (for the removal of the bulk of the ethane and any other materials of low volatility), and thence into a liquid air trap. There followed ten bulb-to-bulb fractional distillations in which large fore and after fractions were discarded. The best fraction finally collected assayed 100% pure on the basis of the volume contraction consequent upon combustion with excess oxygen, and also on the basis of the carbon dioxide produced in such combustion.

Several synthetic test mixtures were made up from the pure gas samples and used to test the satisfactory operation of the apparatus as a whole, and the validity of the fractional combustion in particular. The results obtained, as shown in Table I, were gratifying in both respects.

ACKNOWLEDGMENT

It is a pleasure to acknowledge the continued advice and assistance of Gregory P. Baxter in the conduct of this research.

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Determination of Nicotine in the Air

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A method for detemining nicotine in the air, existing either in the form of tobacco dust or as free vapor, has been outlined. The method consists of collecting the dust or vapor by means of an impinger, freeing the nicotine (in the case of dust) by alkalizing, developing a characteristic color with β-naphthylamine and cyanogen bromide in the presence of acetate, and suitably measuring the intensity of the color so produced. Optimum conditions of pH, temperature, time of development, concentration, and volume of reagents were determined. Data are also given on the extent of potential interfering compounds. A minimum concentration of 0.25 microgram of nicotine per milliliter of solution can be determined by the method outlined with a cell depth of 2 cm.

A SEARCH of the literature reveals the absence of any satisfactory method for the determination of nicotine in the air. In order properly to evaluate either existing or potential health hazards in industrial establishments, where workers are exposed to either nicotine vapors or tobacco dust, this determination is necessary. Moskalev (*13*) has published a method which involves a differential titration using a mixture of indicators, preceded by lengthy removal of interfering substances and steam-distillation. When dealing with small quantities of either nicotine or dust and with numerous samples—conditions usually encountered in industrial hygiene investigations—the need for a more satisfactory method of determination is obvious.

Nicotine produces a colorimetric reaction with cyanogen bromide in the presence of an aromatic amine. König (*7*) first observed the reaction, which has since been utilized by Barta and Marschek (*3*) and by Markwood (*11*) for the determination of nicotine in tobacco leaf. It was felt that the method as developed by Markwood (*9*, *10*) held promise for industrial hygiene work, and with some modifications could be made to yield satisfactory results.

Nicotine may exist in the air in the form of the vapor itself, or as the chief alkaloid of tobacco dust. In either case, it can be satisfactorily collected by the impinger. This device has been described by Bloomfield and DallaValle (*4*), Jacobs (*6*), and others. In its use by the authors for nicotine, distilled water was used as the collecting liquid.

REAGENTS AND APPARATUS

Impinger. Standard Pyrex all-glass graduated model as described by DallaValle (*5*) and sold commercially by laboratory supply houses. A source of suction, with a suitable flow-measuring device, to operate the impinger at a rate of flow of 1 cubic foot per minute is needed.

Centrifuge. Any commercial model on which 15-ml. tubes can be used and which can be operated at 3000 r.p.m. is satisfactory.

Spectrophotometer (or photoelectric colorimeter). Any commercial model on which the measurement can be satisfactorily made at 490 mμ with necessary cells (or tubes) and other accessories. In this investigation an Evelyn photoelectric colorimeter (Rubicon Co., Philadelphia, Pa.) was used for all readings except the S-T data, which were obtained on a Coleman spectrophotometer, Model 11.

Centrifuge Tubes, 15-ml. Pyrex, graduated in 0.1 ml.

Chemical Glassware. Graduated pipets, 1-, 5-, 10-ml. sizes, all graduated in 0.1 ml.; volumetric flasks and reagent bottles.

Microburet.

Cyanogen Bromide. Six grams of reagent grade cyanogen bromide (Eastman No. 919) dissolved in each 100 ml. of 95% (redistilled) ethyl alcohol.

Caution. The vapors of cyanogen bromide are extremely irritating and toxic and should not be inhaled. The solid reagent readily vaporizes at room temperature. It should therefore be thoroughly cooled in the refrigerator before being dissolved, and the weighing and dissolving should be done under an exhaust hood. The solution keeps well in a dark bottle at room temperature, but for use it is desirable that it be cold. It is therefore recommended that it be kept in the refrigerator. When stored, it keeps almost indefinitely.

β-Naphthylamine. Reagent grade β-naphthylamine (0.6 gram) dissolved in each 100 ml. of 95% (redistilled) ethyl alcohol. The solution should be stored in a dark bottle and kept in the refrigerator to ensure its being at a constant cold temperature prior to use.

Sodium Hydroxide, 30% solution. Thirty grams of sodium hydroxide per 100 ml. of distilled water.

Acetic Acid. Two aqueous solutions: 1 to 20 and 1 to 2000 made by diluting glacial acetic acid.

Alcoholic Potassium Acetate. Two grams of the salt dissolved in 100 ml. of 95% (redistilled) ethyl alcohol. This solution should be kept in the refrigerator to ensure its being at a constant cold temperature prior to use.

Phenolphthalein Indicator. Usual 0.04% alcoholic solution.

Standard Nicotine. An accurately weighed portion (20 to 25 mg.) of reagent grade nicotine (Eastman No. 1242) is sealed into a small thin-walled glass bulb. This is broken under water into a 50-ml. glass-stoppered flask, the volume of water being equal in milliliters to the weight of nicotine in milligrams. Thus, 1 ml. of this solution contains 1 mg. of nicotine. The required volume of water is accurately added from a graduated pipet or buret with the last few drops rinsing the stirring rod used for breaking the bulb. Ten milliliters of this solution are made up to 500 ml. with distilled water in a 500-ml. glass-stoppered volumetric flask. This final solution contains 20 micrograms of nicotine per milliliter and is used for preparing the standards. Both solutions will keep for some time (at least 2 months) in the dark without any noticeable depreciation in strength.

Distilled Water (adjusted pH). For making up to volume the standards used in preparing the calibration curve, distilled

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water should be neutralized (with potassium hydroxide and acetic acid) just to the disappearance of the phenolphthalein end point, thus ensuring pH of the standard solutions within the proper range.

PROCEDURE

The contaminated air is drawn through the impinger in the usual manner at a rate of 1 cubic foot per minute. The volume of air sampled should be such that the total quantity of nicotine collected is at least 50 micrograms. If protected from light, the field samples can be kept for several days without any noticeable deterioration in nicotine content.

Sufficient distilled water is added to the impinger to bring the total volume to the 100-ml. mark. After thorough shaking, a 10 to 11-ml. aliquot is removed to a graduated (15-ml.) centrifuge tube. The exact volume of the aliquot is noted, 0.1 ml. of 30% sodium hydroxide is added, and the tube is stoppered and occasionally shaken during a 10- to 15-minute period. The alkali releases free nicotine from the tobacco dust. (If free nicotine vapor is being determined, this step can be eliminated. The solution should, however, be carefully neutralized and centrifuged as described below.) At the end of that time, 2 drops of phenolphthalein indicator are added and the solution is carefully neutralized with acetic acid to the disappearance of any pink color. The neutralization is accomplished with the 1 to 20 solution until the end point is approached and then carefully completed with the 1 to 2000 solution, using a small-bore pipet. An excess of acid must be avoided. The volume is read accurately to the nearest 0.1 ml. The tube is then centrifuged for at least 20 minutes at approximately 3000 r.p.m. At the end of this time all the suspended dust should be deposited in the bottom of the tube. If not, additional centrifuging is necessary.

Exactly 10 ml. are carefully pipetted from the centrifuge tube and transferred to the colorimeter tube. The volume in the centrifuge tube should be at least 11 ml. for ease of removal of the aliquot. Two milliliters of the alcoholic potassium acetate solution and 4 ml. of the β-naphthylamine reagent are added. The tube is shaken to ensure thorough mixing, the transmission measured (to correct for any color resulting from the tobacco as described below), and 1 ml. of the cyanogen bromide solution added. The tube is again thoroughly shaken and then placed in the dark for development of the color for 30 minutes at 22° to 28° C. In order to minimize temperature variations and to obtain uniform results, the potassium acetate, β-naphthylamine, and cyanogen bromide reagents should be cooled to some constant temperature, say 5° C., and used at this temperature. The cooling is also desirable to reduce the heat of dilution which results upon mixing. The light transmission is then measured on a photoelectric colorimeter or spectrophotometer at 490 mμ and the nicotine determined by referring the reading obtained to a previously prepared calibration curve of micrograms of nicotine *vs.* galvanometer readings. A blank tube, containing 10 ml. of distilled water and the same volume of all the reagents as used in the unknown, is used to obtain the 100% transmission reading.

The total quantity of nicotine in the impinger is calculated from the formula:

$$\gamma_T = \frac{10\gamma V_2}{V_1}$$

where γ_T = micrograms of nicotine in impinger
γ = micrograms of nicotine in 10-ml. aliquot as read from curve
V_1 = volume (ml.) of aliquot removed from impinger for analysis
V_2 = volume (ml.) after final neutralization

If the colorimeter or spectrophotometer employed utilizes a transmission cell inadequate in size to accommodate mixing the above volumes of sample and reagents, the solutions can be mixed and developed in suitable volumetric flasks and then transferred to the instrument cell for transmission measurement.

Preparation of Calibration Curve. The curve against which the samples are referred for determination of nicotine content is prepared in the customary manner by adding to four colorimeter tubes 0.25, 0.5, 1.0, and 2.0 ml., respectively, of the standard nicotine solution. This corresponds to nicotine contents of 5, 10, 20, and 40 micrograms. A microburet should be used for the measurement. A sufficient quantity of the distilled water, whose pH has been adjusted as described above, is added to make up each to a total of 10 ml. The reagents and the development of color are identical to those already described for the samples. The transmission readings are plotted on the logarithmic axis *vs.* the nicotine content, in micrograms, on the arithmetic axis of semilogarithmic graph paper. A straight line should result.

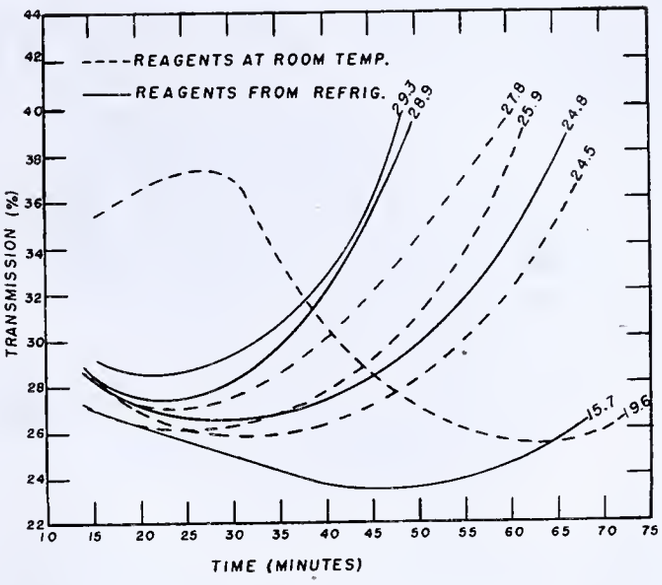

Figure 1. Time and Temperature Effects on Color Intensity

VARIABLES DETERMINING COLOR DEVELOPMENT

Markwood (*10*) has investigated thoroughly the quantitative characteristics of this color reaction, and his investigations have formed the basis of the authors' work. In several cases, however, they have been able to obtain increased sensitivity and more consistent results by changing both the concentration and the order of the addition of some or all of the reagents. The results of their investigations into the several variables and the determination of the optimum conditions entering into the chromogen formation, are described below.

Temperature and Development Time. The effect of temperature and time of development was investigated by using a series of eight solutions, all containing 40 micrograms of nicotine in 10 ml. of distilled water, and all developed as outlined in the Procedure. The environmental temperature at which development occurred was varied by using either small water baths or heated cabinets. The actual temperature of the solution itself is determined not only by the environmental temperature but by the temperature of the reagents. Accordingly, four of the solutions were developed with reagents taken directly from a refrigerator kept at a temperature of 5° C., while the remaining four were developed with reagents at room temperature (28° C.). The temperature of each solution was checked at 15-minute intervals as the color development proceeded and the mean of these values is given on each curve in Figure 1. The transmission was also read at 15-minute intervals, and the results are shown graphically in the figure, with time of development plotted against transmission readings (%).

The curve corresponding to the temperature of 15.7° C. was obtained by development in the refrigerator (5° C.). The solution became turbid, owing to salt crystallization at this temperature. With the exception of this curve, the remaining data indicate that the time required for maximum intensity of color increases markedly as the temperature becomes lower and vice versa, and that the intensity is more greatly affected by temperature changes as the time of development proceeds. This is especially true after 30 minutes. For this reason, because the point of maximum intensity approximates 25 to 30 minutes, depending upon the temperature, and finally because the interference from nornicotine is relatively small at the end of this time, 30 minutes was selected as the proper time of development. By keeping the reagents at a constant temperature in the refrigerator, less variation results in the intensity readings, and

with an environmental temperature of 22° to 28° C. reasonably consistent results can be obtained. If the reagents are not cooled prior to use, sufficient heat of dilution results upon mixing the alcoholic and water solutions to cause an immediate temperature rise with much more rapid fading of color.

pH of Solution. Markwood (*10*) found an optimum pH of 10 for his solutions, but a satisfactory pH at the phenolphthalein end point (pH approximately 8). The authors investigated the effect of varying the pH of the solution (before addition of the β-naphthylamine and cyanogen bromide) upon the color development. The results are shown in Table I.

Table I. Effect of pH on Color Intensity

pH	Transmission	pH	Transmission, %
5.7	14.0	7.4	11.3
5.7	14.3	7.4	11.3
6.8	11.3	9.1	11.3
6.8	11.0	9.1	11.0

These data were procured prior to the establishment of the final procedure, and differ from it only in that 5 ml. of β-naphthylamine instead of the finally adopted 4 ml. were used. All the samples contained 80 micrograms of nicotine in 10 ml. of distilled water. Following the addition of the acetate solution, the pH was varied by using a few drops of very dilute potassium hydroxide and acetic acid and measuring the pH with a glass electrode potentiometer. The color was developed with β-naphthylamine and cyanogen bromide and read in the usual manner. The results indicate no measurable difference in color intensity over the range 6.8 to 9.1 and satisfactory neutralization to the phenolphthalein end point. An excess of acid, however, decreases the intensity of color.

Acetate Concentration. Markwood (*10*) reported that the presence of acetate markedly intensified the color, while the presence of either chloride or sulfate decreased it. These same facts were confirmed by the authors. In some preliminary experiments they observed that tartrate ion will also intensify the color, but to a lesser extent than acetate. They observed no measurable difference in results when using either sodium or potassium acetate, but adopted the potassium salt because of its greater solubility in alcohol. The need for the alcoholic solution is discussed below.

The effect of varying the acetate concentration on color intensity is shown in Table II. All samples contained 40 micrograms of nicotine in 10 ml. of distilled water, and 2 ml. of alcoholic potassium acetate of the indicated concentration. They were developed as outlined in the Procedure.

Table II. Effect of Acetate Concentration on Color Intensity

Sample No.	Concentration of $KC_2H_3O_2$ Used	$KC_2H_3O_2$ Concentration in Final Volume	Transmission
	%	*G./100 ml.*	%
1	0	0	45.0
2	0.5	0.06	39.0
3	1.0	0.12	31.0
4	2.0	0.24	27.0
5	4.0	0.47	31.0

The maximum intensity occurring at or near a concentration of 0.24 gram per 100 ml. agrees with Markwood's value (*10*) (sodium acetate) of 0.2 gram per 100 ml. Additional determinations in the 0.25 region might shift the value somewhat either way, but the small difference in intensity observed between the 0.12 and 0.47 values did not warrant these. Hence 2 ml. of 2% solution were adopted as the final acetate concentration to be used.

Sequence of Addition of Reagents. In the initial phase of this investigation, the cyanogen bromide solution was added prior to the β-naphthylamine, but it was impossible to obtain consistent results. The reason for this inconsistency could not be determined until quite by chance the order of adding these reagents was reversed; reproducible results could then be obtained readily. The time elapsing between the addition of the cyanogen bromide and β-naphthylamine solutions, when this was the order of addition, was an inverse function of the color intensity obtained, the greatest intensity being obtained with the least elapsed time between the additions. No quantitative study was made of this, inasmuch as it was found that the difficulty could be overcome by merely reversing the order in which the two reagents were added to the solution. Sometime after observing this, the authors' attention was called to the work of Lamb (*8*) with nicotinic acid in which he found, when using cyanogen bromide and aniline as reagents, that consistent results were obtained only if the aniline was added prior to the cyanogen bromide. His results differ from the authors', however, in that he found the color intensity increasing as the elapsed time increased, when adding the cyanogen bromide before the aniline.

It is important, therefore, that in all cases the β-naphthylamine solution be added prior to the cyanogen bromide.

Table III. Effect of β-Naphthylamine and Cyanogen Bromide Concentrations on Color Intensity

	Transmission, Per Cent					
	0.3% β-naphthylamine		0.6% β-naphthylamine		0.9% β-naphthylamine	
CNBr, Ml.	4 ml.	5 ml.	4 ml.	5 ml.	4 ml.	5 ml.
1 (1%)	50.0	50.0	53.5	60.0	Turbid	64.0
1 (4%)	30.8	33.3	28.8	31.0	Turbid	36.3
1 (6%)	31.3	31.3	25.0	28.3	Turbid	30.8
1 (8%)	36.5	34.5	30.0	32.3	Turbid	32.0

Concentrations and Volumes of Cyanogen Bromide and β-Naphthylamine. The concentrations of both cyanogen bromide and β-naphthylamine were believed to influence the intensity of color. Likewise, the final volume of solution determines inversely the concentration of chromogen. The use of a 10-ml. aliquot of sample appeared to be most logical from the standpoint of representative sampling, sensitivity, and suitability for centrifuge tubes. A minimum volume of aliquot was desired, inasmuch as additional determinations were frequently required on the same impinger sample. Accordingly the effects of varying the concentrations of both cyanogen bromide and β-naphthylamine, and the volume of the latter, were studied, with the results shown in Table III.

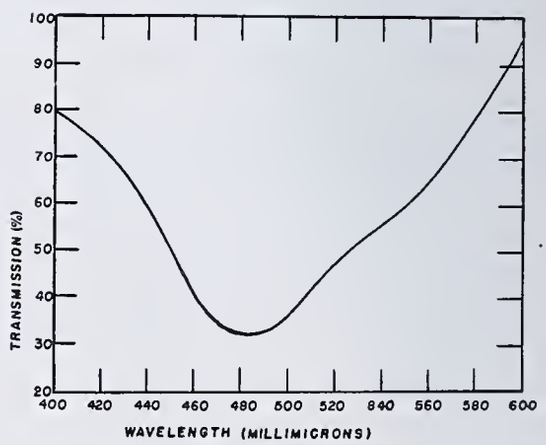

Figure 2. Spectral Transmission Curve for Nicotine

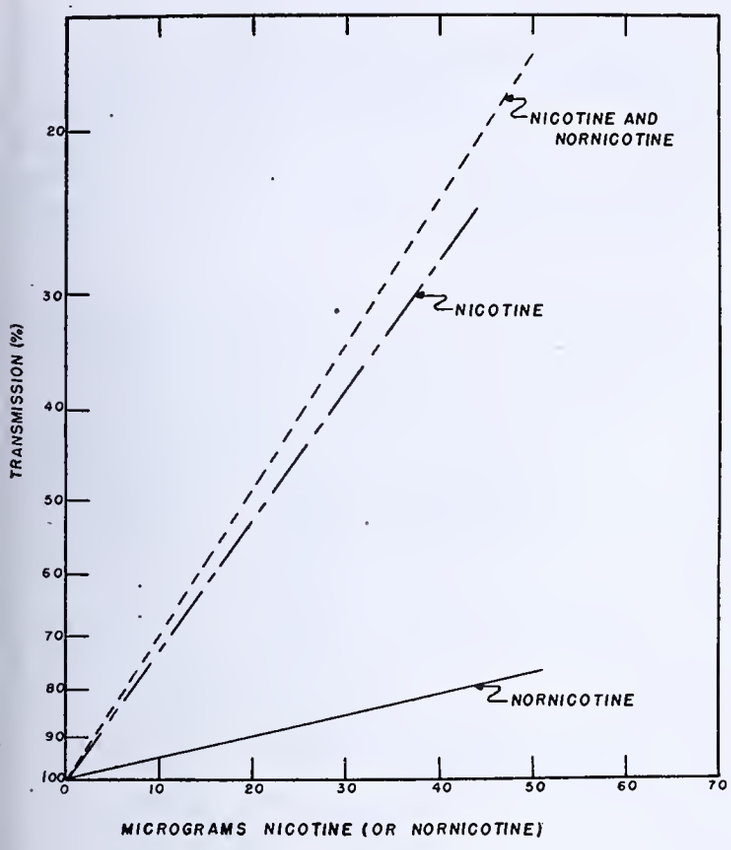

Figure 3. Interference of Nornicotine

All the previously mentioned solutions contained 40 micrograms of nicotine in 10 ml. of distilled water (the pH of which had been adjusted as described under Procedure to the phenolphthalein end point), 1 ml. of alcoholic potassium acetate reagent, and the indicated volumes of β-naphthylamine and cyanogen bromide. In all cases the percentage concentration refers to the weight of the amine in grams, equal to the indicated percentage, dissolved in 100 ml. of 95% ethyl alcohol. The colors were developed in the usual manner as outlined in the Procedure.

An inspection of Table III reveals that the maximum intensity of color results from the use of 1 ml. of 6% cyanogen bromide and 4 ml. of 0.6% β-naphthylamine with the volume of aliquot used (10 ml.). Markwood (*9*, *10*) used a cyanogen bromide solution approximating 0.8% and a β-naphthylamine solution of 0.2%. The data in Table III show the concentrations recommended by the authors to be considerably better for obtaining increased intensity. Minimum nicotine concentrations of 0.25 microgram per ml. can be estimated with a light transmission path of 2 cm. (7/8 inch test tube). This is believed to be the most sensitive means yet developed for determining nicotine. Markwood's (*9*) minimum value, although numerically the same as the authors', was attained with a much greater thickness (10 cm.) of liquid layer.

In the initial phases of this investigation, it was observed that turbidity of the samples depended not only upon the reagent concentrations but upon the final alcoholic concentration and final volume of the solution, with increasing alcoholic concentrations and total volumes decreasing the turbidity. Accordingly, in order to keep the final volume to a minimum and to prevent turbidity, alcoholic rather than aqueous reagents were used. Even with the volumes of reagents finally standardized upon, turbidity will result upon several hours' standing. The 4-ml. β-naphthylamine and the 2-ml. potassium acetate volumes are the minimum which can be used to prevent turbidity.

SPECTRAL TRANSMISSION DATA FOR NICOTINE

The usual type of S-T (spectral wave length *vs.* per cent transmission) curve was determined, with the results shown in Figure 2. A Coleman Model 11 Universal spectrophotometer was used for making the determinations. The curve shows a minimal transmission at a wave length of 480 to 490 mμ.

Table IV. Effect of Insecticides on Color Intensity

Insecticide	% Transmission
None (nicotine only)	28.3
Bordeaux mixture	28.5
Lime-sulfur	27.5
Lead arsenate	28.0
Cryolite	27.5
Paris green	28.5
Calcium arsenate	27.8

INTERFERING SUBSTANCES

Interfering substances of interest in this investigation were the various insecticides used on tobacco plants and nornicotine. The latter compound is an alkaloid—similar in many respects to nicotine—found in varying degrees in tobacco. Markwood (*11*, *12*) has reported it to be the predominating alkaloid in certain types of tobacco. Accordingly, the extent to which it and the various insecticides interfered in the method of analysis was determined.

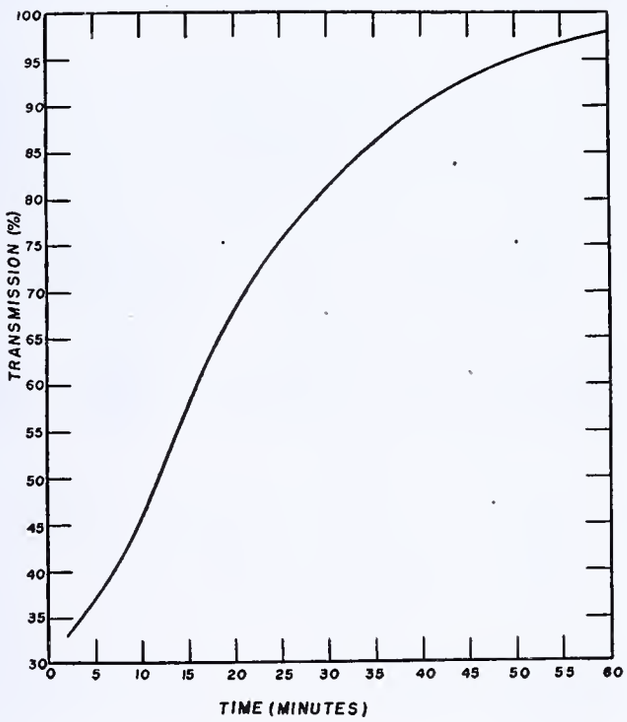

Figure 4. Effect of Developmental Time on Nornicotine Color

The insecticides used were lead arsenate, calcium arsenate, cryolite (synthetic), lime-sulfur, Paris green, and Bordeaux mixture. All were of the commercial grade normally sold for insecticidal uses. The nornicotine was from a sample of the pure alkaloid obtained from the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, and was predominantly the *l*-stereoisomer. Except for the varying times as noted, all results were obtained by developing the solutions as outlined in the Procedure. In the case of nornicotine, transmission curves of per cent transmission *vs.* concentration (micrograms) were determined for solutions containing only nornicotine, in amounts from 5 to 50 micrograms, and for solutions containing both nicotine and nornicotine, each in amounts from 5 to 50 micrograms. The results are shown in Figure 3 in comparison with a similar curve for solutions containing only nicotine. All these results were obtained by reading the transmissions at the end of 30 minutes' development time.

The nornicotine color fades very rapidly. This is shown in the curve of Figure 4 where the transmission *vs.* time of development of a solution containing 40 micrograms of nornicotine is plotted.

This curve shows clearly that by choosing a proper time of development, the interference produced by nornicotine can be almost completely eliminated. By reading the transmission at the end of 30 minutes' development (the optimum time for nicotine), the results will be approximately 10 to 12% high when the two alkaloids are present in equal amounts (see Figure 3).

The results of Table IV are the average of duplicate samples, all developed for 30 minutes in the usual manner. All samples contained 40 micrograms of the indicated insecticide and 40 micrograms of nicotine.

The results show no significant difference in any of the solutions. They are similar to those obtained by Markwood in his insecticide method, except that he found a significant interference resulting from lime-sulfur.

DISCUSSION AND NOTES

Extraction of Nicotine. The standard A.O.A.C. method (*1*) of determining nicotine in tobacco leaf involves a steam-distillation of the nicotine from alkaline solution, followed by gravimetric determination with silicotungstic acid. In order to dispense with the steam-distillation procedure, the possibility of extracting the nicotine from tobacco dust by merely alkalizing the aqueous suspensions was investigated.

Samples of settled atmospheric dust collected from tobacco stemming plants were used. The dust was sieved through a 150-mesh screen, with that portion passing through being used for analysis. Simultaneous determinations of the nicotine content of screened sample were made by the modified steam-distillation technique of Avens and Pearce (*2*), and by alkaline and water extractions as noted in Table V. The extractions were made by simply adding either the alkaline or water solutions to a 15-ml. centrifuge tube containing a weighed quantity of the dust, allowing it to stand with occasional shaking for approximately 10 minutes, then centrifuging, neutralizing, and developing the color as described in the Procedure.

In the case of the steam-distillation, the distillate was carefully neutralized to the phenolphthalein end point with potassium hydroxide, a suitable aliquot taken, and the nicotine color developed in the usual manner.

Inasmuch as the distillate was collected in dilute hydrochloric acid, a separate calibration curve, in which the standards contained the same quantity of potassium chloride as the steam-distillate aliquots, was prepared for reading the nicotine content. To ensure complete nicotine evolution, 250 ml. of distillate were collected instead of the 100 ml. as suggested by Avens and Pearce (*2*). By doing this the authors were able to evolve and collect in the 250 ml. 98 to 99% of the total nicotine in the sample, as shown by the analysis of successive 100-ml. portions of distillate. The water used for the water extractions had been adjusted to the phenolphthalein end point prior to the extraction.

These results indicate that the alkaline extraction method is approximately 95% as efficient as the steam-distillation procedure on atmospheric tobacco dust. This is certainly satisfactory for field determination.

Volatilization of Nicotine by Aeration. To determine whether any appreciable loss of nicotine occurs by volatilization by continuous bubbling of air through the solution, such as occurs in the impinger, four impingers were made up with an aqueous nicotine solution (1 mg. of nicotine in 100 ml. of water in each), and room air was drawn through each for 30 minutes at a rate of 1 cubic foot per minute. The average loss of nicotine of all four was 1.5%.

Correction for Color from Tobacco. If the tobacco dust concentration is appreciable, a yellow to light brown coloration will result with the alkaline extraction. A correction for this must be made before developing the nicotine color. This can be done by reading the transmission just prior to the addition of the cyanogen bromide reagent and making the necessary correction by using the L-G (chromogen concentration *vs.* galvanometer reading) table accompanying the colorimeter.

It should be remembered that the volume of the solution changes from 16 to 17 ml. upon the addition of the cyanogen bromide. This increase in volume must be taken into account when making the correction.

Table V. Efficiency of Various Methods of Nicotine Extraction

Sample No.	Method	γ Nicotine per Gram Sample of Dust	% Nicotine Recovered (Steam Distillation = 100%)
1	Steam distillation	3695	100
	Water extraction	3005	81.3
	0.05% NaOH extraction	3400	92.0
	0.10% NaOH extraction	3380	91.5
	0.30% NaOH extraction	3540	95.7
2	Steam distillation	3787	100
	Water extraction	2970	78.4
	0.05% NaOH extraction	3600	95.2
	0.10% NaOH extraction	3420	90.2
	0.30% NaOH extraction	3420	90.2
3	Steam distillation	2890	100
	Water extraction	2200	76.3
	0.05% NaOH extraction	2820	97.5
	0.10% NaOH extraction	3010	104.0
	0.30% NaOH extraction	2880	99.6

Average % nicotine recovery

Water extraction	78.7
0.05% NaOH	94.9
0.10% NaOH	95.2
0.30% NaOH	95.2

ACKNOWLEDGMENTS

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Nitrogen Determination by the Micro-Dumas Method

Improvements in Apparatus

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Improvements in apparatus for the Dumas nitrogen microdetermination are described. The original microazotometer of Pregl has been modified by replacing the top stopcock with a ball and socket valve, which requires no lubricant for its operation. Elimination of the tapered surface of contact prevents freezing by the potassium hydroxide and the absence of lubricant precludes errors due to contamination from that source. Pure carbon dioxide for the analysis is produced from dry ice in a modified Hershberg and Wellwood generator. Precise control over combustion of sample is effected by a specially designed chimney for the movable burner.

THE extensive use of Pregl's (*5*) micro-Dumas method for determining nitrogen in this laboratory has led to several improvements in the original equipment. These changes include use of a specially designed azotometer with ball and socket valve to replace the stopcock-type azotometer, a carbon dioxide generator utilizing solid carbon dioxide as the source of gas, and a special lamp chimney for use in connection with the movable burner. For heating the permanent filling of the combustion tube, a standard electric microfurnace has been found desirable. A photograph of the assembled equipment is shown in Figure 1.

MICROAZOTOMETER

The standard Pregl-type azotometer (*6*) with stopcock at the top of the graduated column introduces errors in the analysis for the following reasons:

Lubricant from the stopcock gets down into the graduated column and sticks to the walls, causing incorrect volume readings. This is due not only to the bulk of the lubricant itself but also to the lubricant's preventing free drainage of the potassium hydroxide from the walls of the column.

The potassium hydroxide attacks the walls of the stopcock, causing it to stick and eventually to leak.

With the exception of methods by which the quantity of nitrogen is determined indirectly by weighing displaced mercury (*1*) or water (*2*), few major changes have been made in the original azotometer introduced by Pregl (*5*). Milner and Sherman (*4*) describe an azotometer designed without a stopcock at the top of the graduated column. It consists of a cup arrangement attached to the top of the graduated column with a glass rod ground into the azotometer tube. Ceresin is applied to the ground surface to prevent sticking. This apparatus, although superior to the original design of Pregl, still has the disadvantages inherent in those requiring wax or grease on the ground joint to prevent freezing.

By replacing the top stopcock of the Pregl azotometer with a vertical ball and socket valve, a nitrometer has been devised which eliminates all the disadvantages of the stopcock-type closure and operates without the use of wax or lubricant other than the potassium hydroxide itself.

The spherical surface of contact favors separation, and freezing by the alkali does not take place, owing to the absence of the wedging action inherent in tapered type closures. The ball and socket valve, which is completely immersed in the alkali, allows the entire surface of contact to be wet evenly with the alkali and channeling is prevented. This is not the case with the stopcock-type arrangement, since the plug of the latter is wet by the alkali only along that portion of its surface which traverses the openings to the graduated column below and the funnel above. The corrosion thus localized in that section causes leakage after the azotometer has been in use for an extended period of time.

The error in volume caused by corrosion of a ball and socket closure would, theoretically, be less than that of the vertical tapered stopper arrangement of Milner and Sherman; in the case of the spherical joint, the rest position of the ball would change only to the extent of the depth of corrosion, since the effective surface of contact is practically horizontal, whereas with the tapered stopper arrangement the surface of contact is almost vertical and a small amount of corrosion would cause a relatively large change in the rest position of the stopper.

The closed bulb arrangement above the opening at the top of the graduated column instead of the open funnel (*6*) or cup

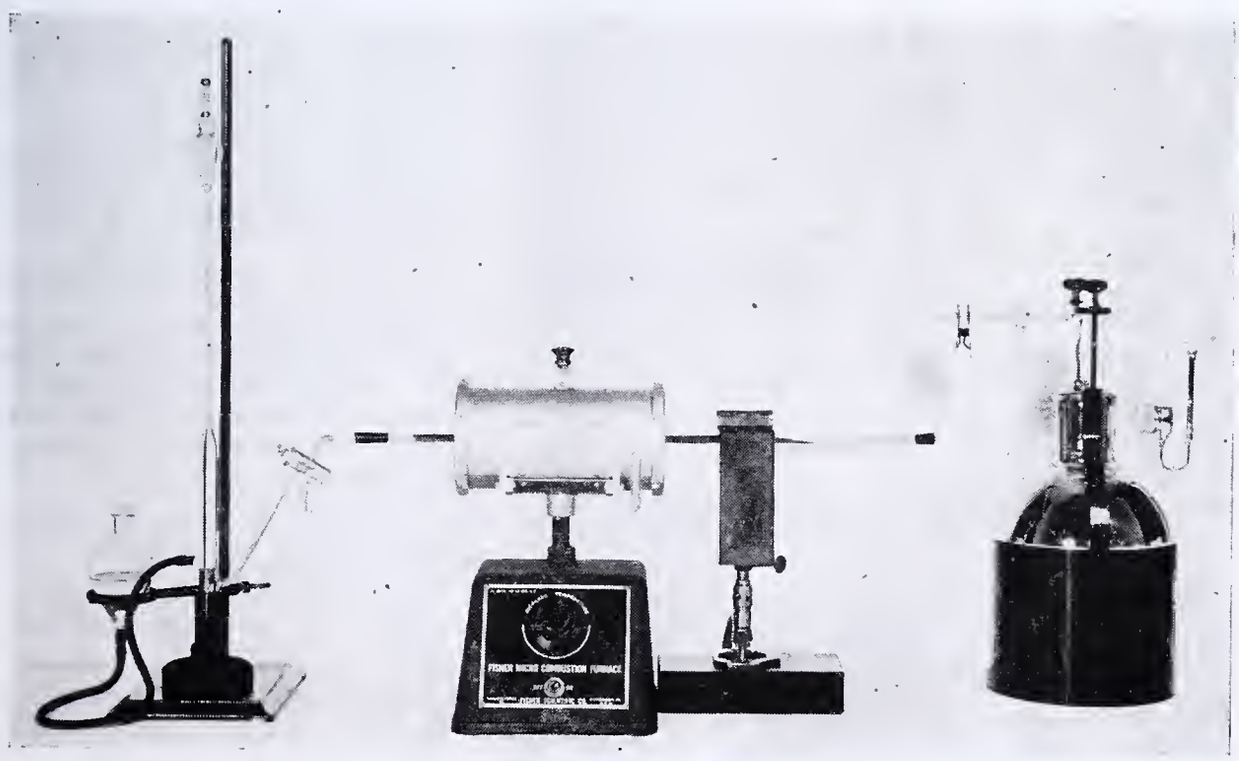

Figure 1. Assembled Equipment

Table I. Reliability of Azotometer after Extended Service, as Shown by Analysis of Pure Acetanilide

Period of Service of Azotometer at Time of Test	Nitrogen Content, % Found		Calculated
Initial	10.38	10.43	10.36
Ten months later	10.41	10.37	10.36

(*4*) eliminates spilling or dust contamination of the potassium hydroxide.

Pyrex has been used in fabricating the azotometer, since it has shown much greater resistance to the action of alkali than soda glass.

Table I gives results of the analysis of a sample of pure acetanilide carried out when the azotometer was initially put into service and after approximately 10 months of continuous use which the instrument had had at the time this paper was submitted for publication. The close agreement of these values shows that the instrument does not lose its reliability after extensive service. The azotometer also showed no measurable leakage when tested after the 10-month period of service. The author's experience with the conventional azotometer indicates the latter to have a much smaller average leakproof period of operation.

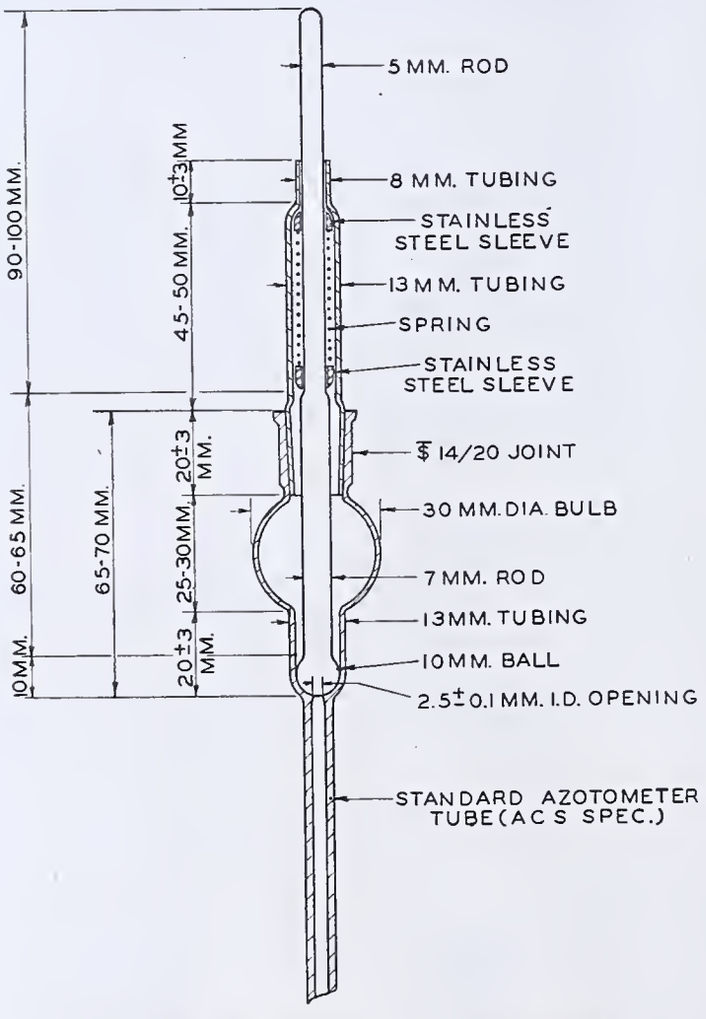

Figure 2. Ball and Socket Type of Azotometer

The upper section of the new azotometer, shown in Figure 2, consists of a plunger actuated by a loosely fitted spring of No. 21-gage music steel wire, approximately fifteen turns in length. This spring is held in place by two stainless steel sleeves of 5-mm. length, one of which presses against a shoulder on the plunger, the other against the constricted top of the spring housing. The spring housing is held in place by sealing its standard-taper joint into that of the azotometer proper with Krönig's cement. When necessary, the apparatus can easily be disassembled for cleaning,

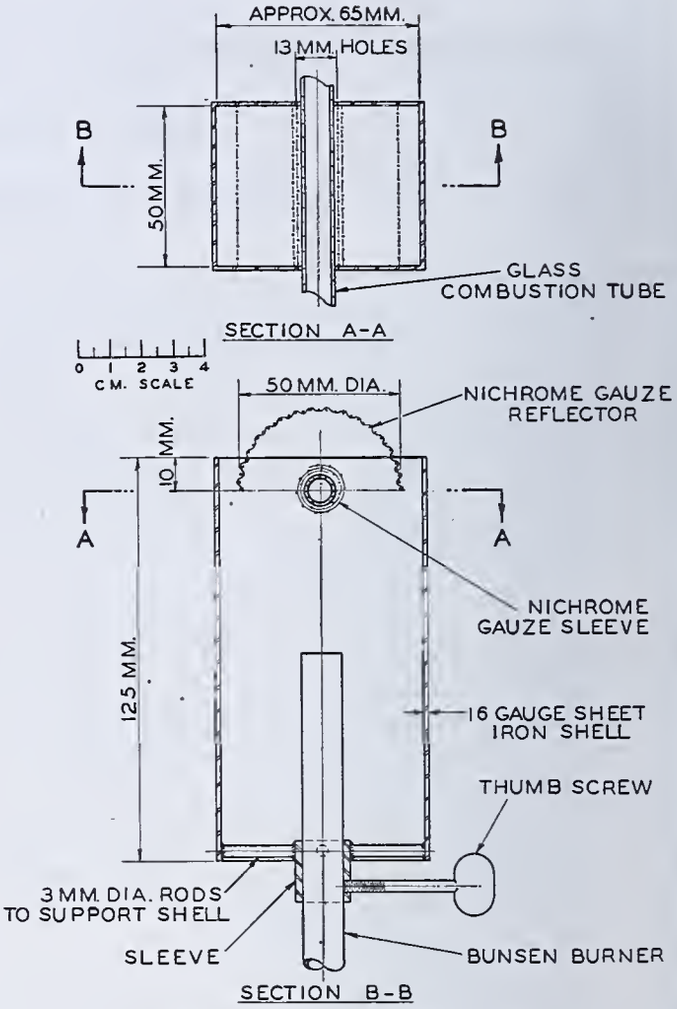

Figure 3. Movable Burner Chimney

by simply reheating the cemented joint. The opening in the top of the graduated column is 2.4 to 2.6 mm. in diameter and tapers gradually to the inside diameter (3.3 to 3.6 mm.) of the graduated column.

When designing this apparatus, it was considered desirable to follow where possible the recommended specifications set forth by the Committee for Standardization of Microchemical Apparatus (*6*). However, poor drainage was experienced when the top of the graduated column was constructed as shown in the drawing accompanying these specifications. The author found that a gradual taper from a 2.5-mm. opening at the top of the graduated column to the inside diameter of this tube worked more satisfactorily. The portion of the azotometer below the ball and socket conforms to standard specifications (*6*).

CARBON DIOXIDE GENERATOR

Of the various methods for supplying pure carbon dioxide gas for the analysis, the use of dry ice has been found most convenient. The gas is most satisfactorily dispensed from a modified Hershberg and Wellwood (*3*) generator which is shown in Figure 1. The principal changes made in the original equipment of these authors include:

Replacement of the paper diffusion disk by a 30-mm. diameter fine porosity Pyrex fritted disk.

Use of a 2000-ml. capacity Dewar flask with standard-taper ground joint instead of the pint-size vacuum bottle.

Addition of a ball and socket connection to the combustion tube.

This apparatus furnishes a continuous stream of pure carbon dioxide gas within 5 hours after filling and lasts for approximately 10 days when a flask evacuated to 6×10^{-7} mm. of mercury is used.

CHIMNEY FOR MOVABLE BURNER

The conventional circular-type movable burner chimney does not give precise control of the combustion of sample in microanalysis because of fluctuations in the flame during heating. A simple unit for this purpose, which also acts as a support for the short Nichrome gauze sleeve and reflector, has been devised and found very effective for all types of microcombustions. The flame is entirely enclosed within the walls of a metal shell and only the portion of the combustion tube within those limits is subjected to the heating.

The construction of the apparatus is shown in Figure 3. It consists of a rectangular sheet-iron shell open at the top and bottom and attached to a Bunsen burner by means of a sleeve and set screw. The sleeve is fastened to the shell by four 3-mm. diameter rods welded to the lower center portion of each face of the shell and to the sleeve. Two holes are drilled on opposite sides of the shell near the top through which the combustion tube is inserted; the Nichrome gauze sleeve is placed around the combustion tube and between the two sides of the chimney. The heat reflector (a semicircular piece of Nichrome gauze, 55 mm. long and 50 mm. in diameter) is notched at the four corners for a distance of approximately 10 mm. in length and 2.5 mm. from the edges to fit into the top of the shell and above the combustion tube.

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Identification of Organic Bases by Means of the Optical Properties of Dilituratates (Nitrobarbiturates)

Primary Aromatic Amines

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The dilituratates of forty-three primary aromatic amines have been prepared and their optical and crystallographic properties recorded. The components of mixtures of isomeric amines may be recognized by a determination of the optical and crystallographic properties of the crystals of the dilituratates prepared from the mixtures.

THE use of the crystallographic and optical properties of the dilituratates has been suggested as a means of identification of organic bases by the authors in a previous paper (*1*), in which they reported the optical constants for the dilituratates of some primary aliphatic amines. The present communication reports similar constants for the dilituratates of primary aromatic amines.

The methods employed are similar to those used in the earlier work. In preparing the dilituratate of a solid amine, the amine was dissolved in hydrochloric or acetic acid and an equivalent quantity of a hot aqueous solution of dilituric acid was added. The amine dilituratate precipitated either immediately or on cooling. Equivalent quantities of dilituric acid and the amine are not important unless dilituric acid itself crystallizes out. If less than an equivalent quantity is added, the dilituratate crystallizes out and the acetate or hydrochloride, being more soluble, remains in solution. Generally speaking, the primary amine dilituratates are less soluble than dilituric acid and so the acid does not complicate the identification, even if added in excess.

The dilituratates were recrystallized from water one or more times. The identity and purity of the salts were established by determination of the nitrogen content by the Kjeldahl method modified to include nitro compounds. The deviation of the percentages found from those calculated did not exceed 0.2 except for the following salts: *p*-bromoaniline, *p*-chloroaniline, and *m*-phenylenediamine, each 0.25; and *o*-nitroaniline, 0.31. Benzidine forms only the monodilituratate, as indicated by the determination of the nitrogen content. The remaining diamino compounds all produce didilituratates, even though the dilituric acid is added in less than equivalent quantities.

The optical data for the dilituratates of forty-three primary aromatic amines are included in Table I. The usual orientation of the dilituratates of *p*-aminophenol, benzidine, *m*-bromoaniline, 2,4-diaminophenol (both forms), 2,4-diaminotoluene, 2,6-dibromo-4-aminophenol, 2,5-dichloroaniline, α-naphthylamine (triclinic), *m*-nitroaniline (from water), *p*-nitroaniline, 3-nitro-4-chloroaniline, *o*-phenylenediamine, *m*-phenylenediamine, and *m*-toluidine does not present a true value for any principal index, but the values can be determined from crushed crystals. The usual orientation of the dilituratates of *p*-aminodiethylaniline, *p*-aminodimethylaniline, 2-amino-5-hydroxytoluene, 2-amino-4-nitrophenol, *o*-aminophenol, *m*-aminophenol (both forms), 3-bromo-4-aminotoluene, *o*-bromoaniline, *p*-bromoaniline (rectangles), *o*-chloroaniline, *p*-iodoaniline, *o*-nitroaniline, and *o*-toluidine permits the determination of one index—β. The crystals of aniline dilituratate (monoclinic) are so oriented that α can be observed. The crystals of the other dilituratates examined are so oriented that the true values for two of the principal indices can be determined—α and β for all except *m*-chloroaniline and *m*-nitroaniline (from alcoholic solution) which show β and γ; and *o*-aminodiphenyl which shows α and γ.

The dilituratates of *m*-aminophenol, aniline, *p*-bromoaniline, 2,4-diaminophenol, and α-naphthylamine exhibit polymorphism. In different preparations the relative proportions of the polymorphs varied, but both forms were always present. When *m*-nitroaniline is crystallized from ethanol the product possesses different properties than those of the compound crystallized from an aqueous solution.

The systems to which the various crystals belong are indicated in column 2 by the letters O, M, and T—orthorhombic, monoclinic, and triclinic, respectively.

The dilituratates of many of the aromatic amines are so flattened that when suspended in a liquid nearly all crystals assume the same or nearly the same orientation. In Table II some optical and crystallographic values are recorded which were observed on the most frequently occurring orientations. The optical orientation designated as acute, obtuse, or optic normal indicates that a centered figure is observed in the usual position of the crystal. The term "inclined" indicates that the interference

figure is not centered. Some crystals show slight variations in orientation, so that the values for the extinction angle and the refractive indices are slightly different on different crystals. Such values are recorded as variable. Many of the crystals present, in their usual orientations, refractive indices which cannot be satisfactorily determined within narrow limits. These indices are also reported as variable.

In Figures 1 to 4 are diagrams of the crystals showing front views as the crystals appear in their most frequently occurring orientations, side views, and in some cases top views. Side and top views were obtained by rolling the crystals in Canada balsam. Extinction angles were all measured between the long direction of the crystal in its particular orientation and the position of nearest extinction. Dotted lines indicate vibration directions and apparent refractive indices are recorded for those crystals which show constant values. An asterisk is used to indicate the higher refractive index in an orientation unless a value is given for it. Observed crystal angles (determined microscopically) which occur consistently are indicated at the corners of the diagrams. Dilturates of 2-amino-1,3-dimethylbenzene, 2-amino-1,4-dimethylbenzene, and β-naphthylamine exhibit two habits; hence two sets of drawings are presented for each of these compounds. Each of those dilturates showing polymorphism (mentioned above) also has two sets of drawings. The various properties recorded in the drawings identify the forms—e.g., one form of *m*-aminophenol has a 135° crystal angle, whereas the other form possesses a 112° angle.

Some of the amine dilturates, when crystallized from water, do not always form large enough or sufficiently well-defined crys-

Table I. Optical Properties of Some Primary Aromatic Amine Dilturates

Amine	System	Extinction Angle	Optic Sign	Refractive Indices Alpha	Beta	Gamma	Elongation	Dispersion
2-Aminocymene	O	Parallel	−	1.507	1.648	1.679	−	$v > \rho$
p-Aminodiethylaniline	M	22	−	1.455	1.659	1.744	±	$v > \rho$
p-Aminodimethylaniline	M	42	−	1.462	1.668	>1.785	+	$v > \rho$
2-Amino-1,3-dimethylbenzene	M	20	+	1.583	1.670	>1.785	−	$\rho > v$
2-Amino-1,4-dimethylbenzene	M	28	+	1.572	1.657	1.752	−	$\rho > v$
4-Amino-1,3-dimethylbenzene	O	Parallel	−	1.522	1.671	>1.785	−	$v > \rho$
5-Amino-1,3-dimethylbenzene	M	39	−	1.543	1.642	1.723	±	$\rho > v$
o-Aminodiphenyl	O	Parallel	−	1.461	1.768	>1.785	−	$\rho > v$
p-Aminodiphenyl	M	12	+	1.603	1.653	>1.785	−	$\rho > v$
2-Amino-5-hydroxytoluene	M	7	−	1.520	1.651	1.763	−	$v > \rho$
2-Amino-4-nitrophenol	M	16	+	1.672	1.722	>1.785	±	...
o-Aminophenol	M	29	+	1.618	1.673	>1.785	±	$\rho > v$
m-Aminophenol (135° angle)	T	26	−	1.501	1.689	>1.785	±	$v > \rho$
(112° angle)	M	33	−	1.480	1.750	>1.785	±	$v > \rho$
p-Aminophenol	T	22	−	1.508	1.715	>1.785	−	$v > \rho$
Aniline	M	16	−	1.535	1.659	1.772	−	$v > \rho$
	O	Parallel	−	1.447	1.738	>1.785	−	$v > \rho$
Benzidine	T	37	+	1.571	1.684	>1.785	−	$\rho > v$
3-Bromo-4-aminotoluene	M	19	+	1.578	1.679	>1.785	−	$\rho > v$
o-Bromoaniline	M	35	+	1.580	1.647	1.788	−	$\rho > v$
m-Bromoaniline	M	16	−	1.525	1.708	1.735	±	$v > \rho$
p-Bromoaniline								
Rectangular	M	18	+	1.456	1.749	>1.785	−	$\rho > v$
Diamond-shaped	M	21	+	1.597	1.688	>1.785	±	$\rho > v$
o-Chloroaniline	M	34	+	1.532	1.657	1.789	−	$\rho > v$
m-Chloroaniline	O	Parallel	−	1.554	1.777	>1.785	−	$\rho > v$
p-Chloroaniline	M	22	+	1.575	1.670	>1.785	±	$\rho > v$
2,4-Diaminophenol								
Tabular	T	43	−	1.498	1.695	>1.785	−	$v > \rho$
Acicular	M	17	−	1.508	1.701	>1.785	±	$v > \rho$
2,4-Dianinotoluene	T	35	−	1.470	1.751	>1.785	±	$v > \rho$
2,5-Diaminotoluene	M	17	−	1.513	1.749	>1.785	±	$v > \rho$
2,6-Dibromo-4-aminophenol	T	44	+	1.590	1.653	>1.785	±	$v > \rho$
2,4-Dichloroaniline	M	24	+	1.572	1.660	>1.785	±	$v > \rho$
2,5-Dichloroaniline	T	28	+	1.532	1.700	>1.785	±	$v > \rho$
p-Iodoaniline	M	9	+	1.500	1.751	>1.785	−	$v > \rho$
α-Napbthylamine	M	14	+	1.635	1.684	>1.785	−	$v > \rho$
	T	44	−	1.650	1.783	>1.785	−	$v > \rho$
β-Naphthylamine	O	Parallel	−	1.483	1.758	>1.785	−	$v > \rho$
o-Nitroaniline	M	33	−	1.540	1.714	>1.785	−	$v > \rho$
m-Nitroaniline, from water	T	28	+	1.522	1.650	1.786	+	$v > \rho$
From ethanol	O	Parallel	−	1.562	1.759	>1.785	+	$\rho > v$
p-Nitroaniline	T	36	+	1.615	1.698	>1.785	−	$v > \rho$
3-Nitro-4-chloroaniline	T	25	+	1.605	1.680	>1.785	−	$v > \rho$
o-Phenylenediamine	T	18	−	1.438	1.737	1.772	−	$v > \rho$
m-Pbenylenediamine	T	30	−	1.493	1.683	>1.785	±	$v > \rho$
p-Phenylenediamine	M	25	−	1.501	1.716	>1.785	−	$v > \rho$
Tolidine	M	25	+	1.590	1.705	>1.785	±	$v > \rho$
o-Toluidine	M	38	+	1.552	1.632	1.766	−	$\rho > v$
m-Toluidine	T	42	+	1.567	1.675	>1.785	±	$\rho > v$
p-Toluidine	M	24	+	1.670	1.680	1.734	±	$\rho > v$

Table II. Apparent Properties of Some Primary Aromatic Amine Dilturates from Most Frequently Observed Orientation

Dilturate	Habit	Optical Orientation	Extinction Angle	Refractive Indices	
2-Aminocymene	Foliated	Obtuse	Parallel	1.507	1.648
p-Aminodiethylaniline	Tabular	Inclined obtuse	Parallel	Variable	1.659
p-Aminodimethylaniline	Tabular	Inclined optic axis	Variable	Variable	1.668
2-Amino-1,3-dimethylbenzene	Lath-shaped	Acute	20	1.583	1.670
2-Amino-1,4-dimethylbenzene	Foliated	Acute	28	1.572	1.657
4-Amino-1,3-dimethylbenzene	Tabular	Obtuse	Parallel	1.522	1.671
5-Amino-1,3-dimethylbenzene	Lamellar	Obtuse	39	1.543	1.642
o-Aminodiphenyl	Lath-shaped	Optic normal	Parallel	1.461	>1.785
p-Aminodiphenyl	Lamellar	Acute	12	1.603	1.653
2-Amino-5-hydroxytoluene	Tabular	Inclined obtuse	Parallel	1.527	1.651
2-Amino-4-nitrophenol	Acicular	Inclined optic axis	Parallel	Variable	1.722
o-Aminophenol	Lamellar	Inclined optic axis	Variable	Variable	1.673
m-Aminophenol (135° angle)	Tabular	Inclined optic axis	Variable	Variable	1.689
(112° angle)	Tabular	Inclined obtuse	Parallel	Variable	1.750
p-Aminophenol	Tabular	Inclined obtuse	Variable	Variable	Variable
Aniline, monoclinic	Tabular	Inclined obtuse	Parallel	1.535	1.671
Orthorhombic	Tabular	Obtuse	Parallel	1.447	1.738
Benzidine	Lath-shaped	Inclined optic axis	30	Variable	1.690
3-Bromo-4-aminotoluene	Tabular	Inclined acute	Parallel	Variable	1.679
o-Bromoaniline	Tabular	Optic axis	Variable	Variable	1.647
m-Bromoaniline	Tabular	Inclined obtuse	Variable	Variable	Variable
p-Bromoaniline, rectangular	Tabular	Inclined acute	Parallel	Variable	1.749
Diamond-shaped	Tabular	Acute	21	1.597	1.688
o-Chloroaniline	Tabular	Inclined optic axis	Parallel	Variable	1.657
m-Chloroaniline	Tabular	Acute	Parallel	1.777	>1.785
p-Chloroaniline	Tabular	Acute	22	1.575	1.670
2,4-Diaminophenol	Tabular	Inclined obtuse	Variable	1.502	Variable
	Acicular	Variable	Variable	Variable	Variable
2,4-Diaminotoluene	Columnar	Variable	Variable	Variable	Variable
2,5-Diaminotoluene	Lamellar	Obtuse	17	1.513	1.749

Table II *Contd.*

Dilurate	Habit	Optical Orientation	Extinction Angle	Refractive Indices	
2,6-Dibromo-4-aminophenol	Tabular	Inclined optic normal	44	Variable	Variable
2,4-Dichloroaniline	Lamellar	Acute	24	1.572	1.660
2,5-Dichloroaniline	Tabular	Inclined optic axis	28	Variable	Variable
p-Iodoaniline	Tabular	Inclined acute	Parallel	Variable	1.751
α-Naphthylamine, monoclinic	Lamellar	Acute	14	1.635	1.684
Triclinic	Tabular	Inclined obtuse	Variable	Variable	>1.785
β-Naphthylamine	Tabular	Obtuse	Parallel	1.483	1.758
o-Nitroaniline	Tabular	Inclined optic axis	Parallel	Variable	1.714
m-Nitroaniline, from water	Tabular	Inclined optic normal	3	Variable	Variable
From ethanol	Lath-shaped	Acute	Parallel	1.750	>1.785
p-Nitroaniline	Tabular	Inclined optic normal	17	1.626	>1.785
3-Nitro-4-chloroaniline	Equant	Inclined optic normal	16	Variable	Variable
o-Phenylenediamine	Lath-shaped	Inclined obtuse	6	1.470	1.756
m-Phenylenediamine	Lamellar	Inclined obtuse	7	Variable	1.694
p-Phenylenediamine	Tabular	Obtuse	25	1.501	1.716
Tolidine	Lamellar	Acute	25	1.590	1.705
o-Toluidine	Tabular	Inclined optic axis	Variable	Variable	1.632
m-Toluidine	Tabular	Inclined optic axis	Variable	Variable	Variable
p-Toluidine	Tabular	Acute	24	1.670	1.680

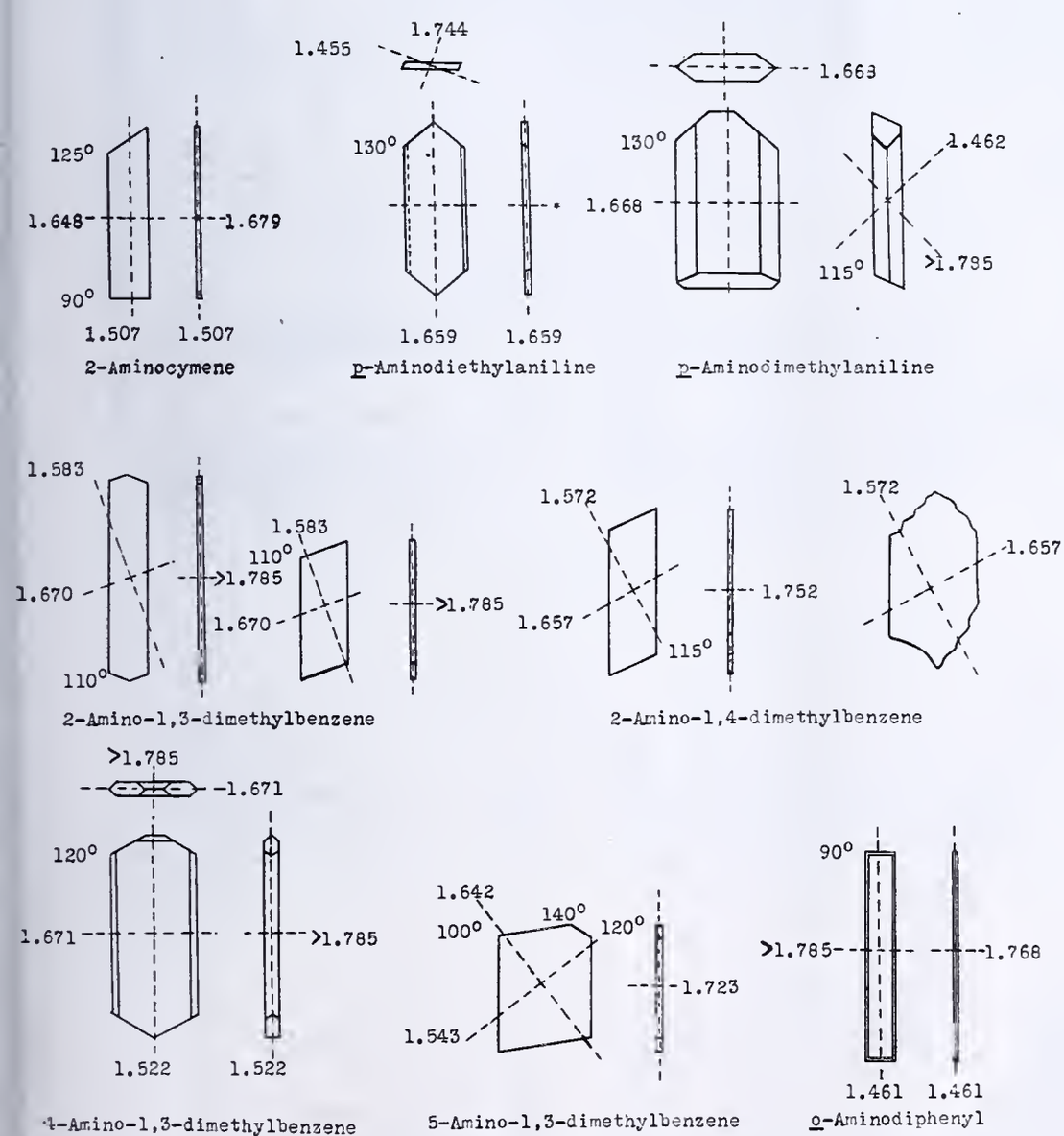

Figure 1. Diagrams of Crystals of Amine Dilurates

tals to make the crystal shape of individual crystals valuable for identification. Attempts at recrystallization from ethanol, ethyl acetate, ether, petroleum ether, and acetic acid failed to produce more regularly formed crystals. Some characteristics of such crystals are the following:

2-Aminocymene forms dendrites, spherulites, and pink masses of crystals. Crystal angles are occasionally 125°, but more frequently 90°. Ends of the crystals are often broken or irregular. 2-Amino-1,4-dimethylbenzene forms some spherulites and masses of crystals, fragments of which show gray polarization colors. Many of the crystals are very thin. 5-Amino-1,3-dimethylbenzene also forms masses of crystals, but fragments show vivid polarization colors. *p*-Aminodiphenyl produces many fragments with dull polarization colors. 2,5-Dichloroaniline crystallizes in masses which show dull polarization colors. α-Naphthylamine forms dendrites along with well-formed crystals. It exhibits polymorphism with the lamellar crystals in greater proportion than the tabular form. *p*-Phenylenediamine often forms masses of very small crystals; larger crystals have 110° crystal angles, but also frequently show broken ends. Tolidine produces masses of crystals which show vivid polarization colors.

Additional descriptions of some of the dilurates will facilitate identification of them. The properties of *p*-aminodiethylaniline dilurate should be taken from freshly prepared crystals or from those which have been properly protected to prevent loss of water of crystallization. Crystals of the dried product retain their shapes nicely, have the appearance of being composed of bundles of needles or fibers, and possess higher refractive indices.

Dilurates of *p*-aminodimethylaniline, *o*-bromoaniline, and *o*-toluidine exhibit no extinction in the usual orientation, but the interference figures (optic axis or slightly inclined optic axis) indicate β to show perpendic-

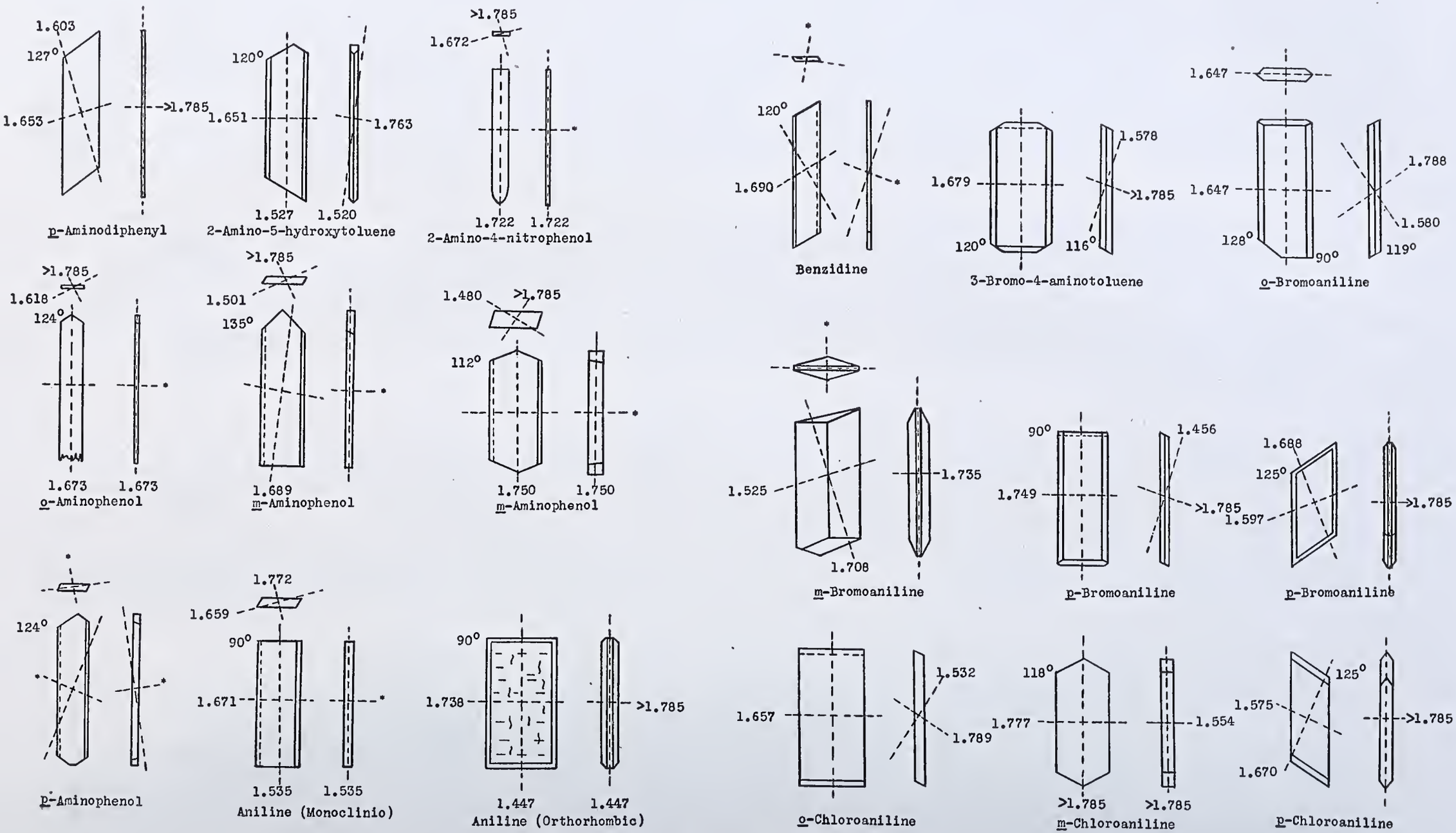

Figure 2. Diagrams of Crystals of Amine Dilturates

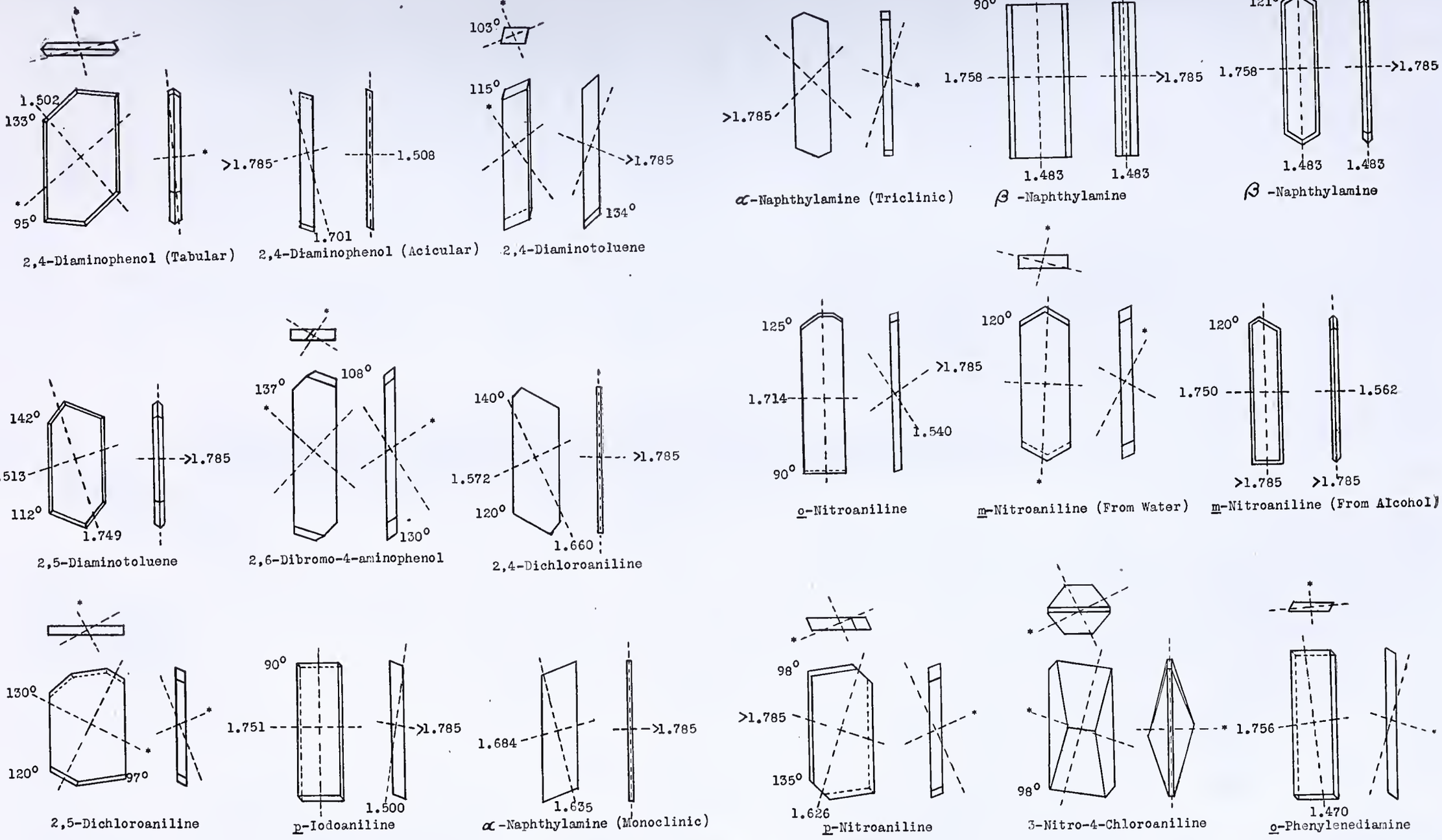

Figure 3. Diagrams of Crystals of Amine Dilturates

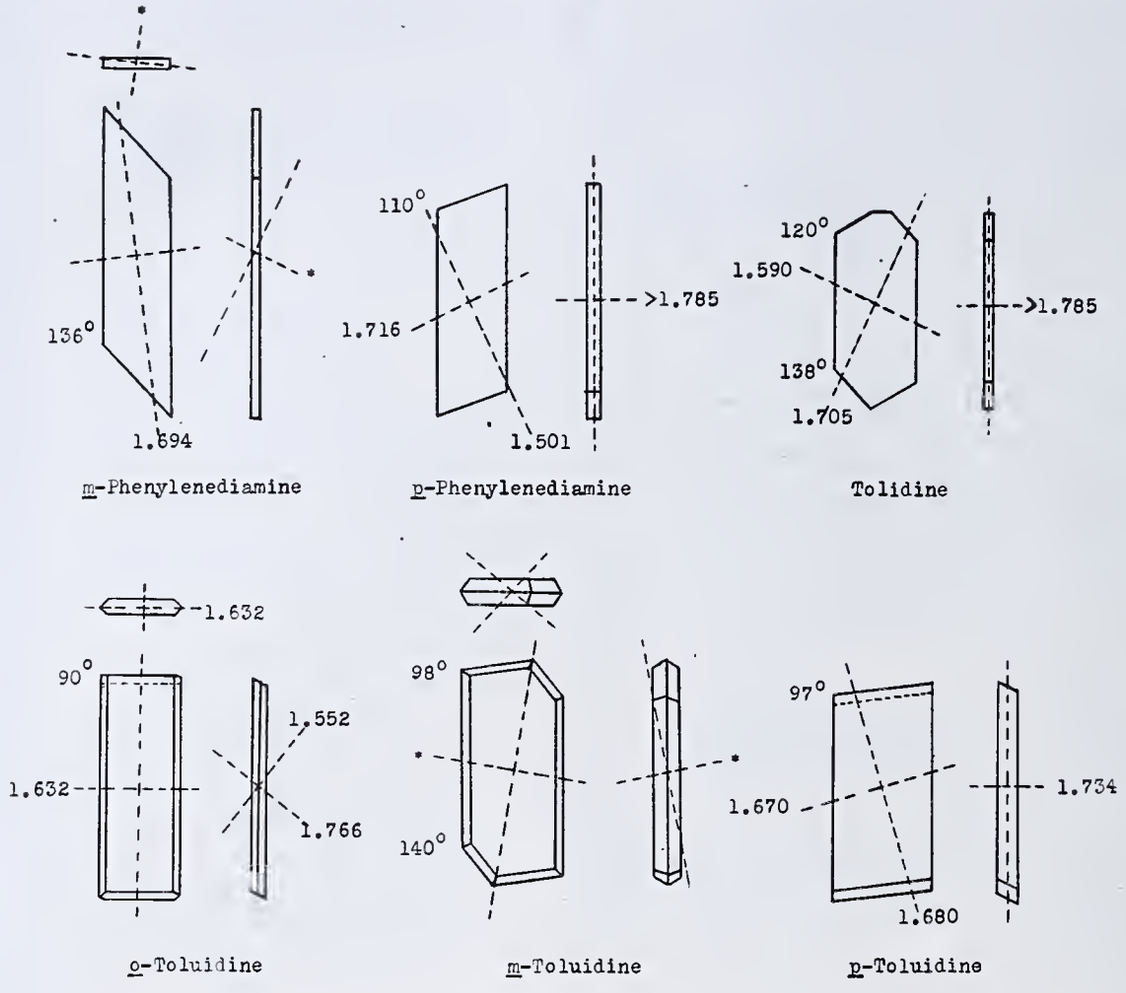

Figure 4. Diagrams o Crystals of Amine Diliturate

ular to the long axis of the crystals. *o*-Aminophenol diliturate also presents no extinction in its usual orientation, but the interference figure (slightly inclined optic axis) indicates that β is parallel to the long axis of the crystal. Many of the crystals of this compound are very thin at the ends and present a broken appearance.

Marked dispersion is shown by *o*-aminodiphenyl diliturate; hence it was necessary to determine β (1.768) with sodium light. With white light the value is approximately 1.774. Crystals of this compound are very long and therefore present many broken ends. *m*-Nitroaniline diliturate, crystallized from ethanol, also presents high dispersion. With sodium light a value of 1.750 is obtained for β and with white light a value approximating 1.762 is found.

Crystals of 2-amino-4-nitrophenol diliturate present a marked inclined dispersion. The isogyre with violet fringe on the convex side seems to be thicker than the other isogyre. Many of the crystals are somewhat rounded and thin at the ends.

Crystals of aniline diliturate, orthorhombic, show cleavage lines in both directions parallel to the outline of the crystal in its usual orientation. The cleavage lines parallel to the long axis of the crystal are somewhat wavy. The orthorhombic crystals are broader than the monoclinic variety. Some samples of aniline diliturate are made up almost entirely of the monoclinic variety, but there are samples in which the orthorhombic form predominates.

When crystals of *m*-bromoaniline, usually fairly large, are lightly crushed, platelets with inclined acute bisectrix figures in the usual orientation are produced. Some of these platelets are so well proportioned that they might be mistaken for another crystal habit. The higher apparent refractive index of the whole crystals lies near 1.708.

Diliturates of *m*-chloroaniline and *p*-toluidine show dispersed birefringence as evidenced by the anomalous polarization colors (purple or blue) at the extinction position.

If crystals of 2,4-diamino phenol diliturate acicular li perfectly flat, then an acut bisectrix interference figure i presented and β (1.701) and (>1.785) show. The crystal are easily rolled in immersio liquids.

Many thin oval-shaped crys tals appear in samples o 2,5-diaminotoluene diliturate.

Yellow crystals of *p*-nitro aniline diliturate slowly de compose to yield colorles crystals. The colorless com pound contains a smaller per centage of nitrogen tha the original colored crystals The colorless crystals have lower refractive indices and a smalle optic angle than the yellow variety.

The usual orientation of *m*-toluidine diliturate shows a lov variable refractive index which is very close to β (1.675). Th crystals are usually fairly large.

It was explained above that many apparent refractive indice are reported as variable. The following are approximate value of some of the apparent refractive indices taken from the crystal in their most frequent orientations. In general these values var between rather wide limits: diliturates of *p*-aminophenol, 1.52 and 1.715; benzidine, 1.607; 3-bromo-4-aminotoluene, 1.602 2,4-diaminophenol (tabular), 1.705; 2,4-diaminotoluene, 1.49 and 1.770; 2,6-dibromo-4-aminophenol, 1.595 and 1.760; 2,5 dichloroaniline, 1.560 and 1.710; *p*-iodoaniline, 1.508; α-naph thylamine (triclinic), 1.780; *m*-nitroaniline (from water), 1.53 and 1.756; 3-nitro-4-chloroaniline, 1.615 and 1.780.

Mixtures of the ortho, meta, and para isomers used in this inves tigation were made and diliturates of the mixtures were prepared The mixtures of crystals of the diliturates exhibited propertie which were characteristic of the individual components of th mixtures. Fractional crystallization of the mixtures resulted in a partial separation which usually simplified the identification Frequently some distinctive characteristic facilitates the recog nition of one of the components—for example, crystals of *m*-chloroaniline diliturate show dispersed birefringence, and they may be readily distinguished from the ortho and para isomers by the anomalous polarization color exhibited by the crystals of th meta compound.

The primary aromatic amine diliturates have been successfully used as unknowns for students in an elementary chemical micros copy course.

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NOTES ON ANALYTICAL PROCEDURES

Modification of the Arsenic Method of Magnuson and Watson

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THE method of Magnuson and Watson (*1*) for the microdetermination of arsenic in biological materials was selected for use in a program concerned with the pharmacology of arsenical drugs and their application to the chemotherapy of certain tropical diseases. Preliminary tests indicated that recoveries were within 5 to 10% of theoretical in the 1- to 10-microgram range, and the method was therefore of the required accuracy. The choice of this method over others which also utilize the molybdenum blue reaction was based chiefly on the rapidity with which samples can be run through. As the authors state, 20 to 30 determinations can be done each day with no difficulty. A simple microstill, commercially available, is the only special apparatus required.

In this laboratory positive blanks and aberrant high values were occasionally encountered in handling this technique. The present paper describes an investigation of the cause of these false readings, and indicates how they can be eliminated by a modification of one of the reagents.

The following steps are carried out in the determination of arsenic in blood, excreta, and organs. Details are described in the original paper (*1*).

The organic material is destroyed in a wet digestion using nitric and sulfuric acid. Perchloric acid may also be used at the end of the process.

Following digestion the arsenic is in pentavalent form in sulfuric acid. This is transferred to the microstill, potassium bromide is added, and arsenic, probably in the form of the pentabromide, is distilled together with a small but significant amount of hydrobromic acid.

The distillate is treated with ammonium molybdate, to form a heteropoly compound with arsenic as the central atom in the complex (*2*).

The heteropole is reduced with hydrazine sulfate to form molybdenum blue, which gives a color with a sharp absorption maximum at 840 mμ. In the final solution, 1 part of arsenic in 25,000,000 may be readily detected and measured, using a Beckman spectrophotometer and a light path of 50 mm.

Woods and Mellon (*2*) found that in the analogous case of phosphorus the hydrochloric acid–molybdate ratio was important. If this ratio was too low, the reaction corresponding to the fourth step would involve reduction of ammonium molybdate itself, to form molybdenum blue even in the absence of the heteropole. Thus, in any method of this type it is necessary to have the acid sufficiently high so that only the heteropole, which contains the element sought, is reduced. Magnuson and Watson (*1*) depended on the hydrobromic acid distilled in the prescribed 4-minute period for this purpose. They state (*a*) that 2.5 to 4.0 milliequivalents of hydrobromic acid are distilled in this time and (*b*) that as low as 1.0 milliequivalent is permissible. In their experiments, as well as the author's, hydrobromic and hydrochloric acid were used interchangeably in the color development steps.

Ten successive distillations were run following the Magnuson-Watson procedure exactly. The distillates were titrated with 0.1 *N* sodium hydroxide using bromothymol blue as indicator. Hydrobromic acid in the distillates was between 1.3 and 3.6 m.e. This range is lower than that given by Magnuson and Watson (*a*, above). The difference is significant, since the experiments recorded in Table I show that a positive blank occurs regularly if less than 2 m.e. of acid is present. Thus, the permissible lower limit of acidity in the original paper (*b*, above) was also subject to revision in the author's hands. Table I indicates that over 2.1 m.e. of halogen acid must be present to avoid formation of molybdenum blue in the absence of arsenic.

In view of these findings it has been possible to eliminate the occasional high values and false positives by the addition of 1 m.e. of hydrochloric acid to the distillate. Since the lowest titer ever found for a distillate was 1.3 m.e., this increment should always be sufficient to bring the total amount of halogen acid to the range where reduction of the simple molybdate and the resulting high values and false positives are completely eliminated. Approximately 500 determinations have been carried out with this modification, and no aberrant results of the type originally found have been observed. The over-all procedure has been found satisfactory with regard to accuracy, speed, and simplicity.

Table I. False Positive Arsenic Readings Due to Low Halogen Acid Concentration in Magnuson-Watson Procedure

(All reagents tested and free of As, Si, and P. Readings from standard As curve, using Coleman Spectrophotometer at 790 mμ.)

Undistilled		Distilled	
Milliequivalents of HCl used	Reading as As, γ	Milliequivalents of HBr found	Reading as As, γ
0	14.5	1.8	1.6
1.0	1.5, 1.7, 2.7	2.0, 2.0	1.0, 2.0
1.5	1.4, 0.6	2.1	1.3
2.0	0, 0, 1.0, 0.6	3.1, 3.1	0
2.5	0, 0, 0	3.5	0
3.0	0, 0, 0	4.0 (5-minute distillation)	0
3.5	0, 0, 0		
4.0			
5.0	0, 0, 0		

MODIFIED PROCEDURE

In order to avoid the handling of an extra solution, the extra milliequivalent of hydrochloric acid is added along with the ammonium molybdate solution. To two volumes of molybdate color reagent, made up according to the directions of Magnuson and Watson, add one volume of *N* hydrochloric acid. In the color development procedure, add 3 ml. of this combined solution instead of 2 ml. of molybdate solution as called for in the earlier paper. All other solutions and directions are unchanged.

SUMMARY

A study has been made of the Magnuson and Watson molybdenum blue method for arsenic in biological materials, which offers certain advantages over others described in the literature. It was found that in their distillation process, insufficient acid was carried over, and the result was occasional high values in the presence of arsenic and positive readings in the absence of arsenic and the other possible interfering elements, silicon and phosphorus. This was ascribed to reduction of simple molybdate ion in insufficiently acid solution, and has been eliminated by addition of hydrochloric acid to the distillate.

ACKNOWLEDGMENT

The author wishes to thank Mildred Salchunas for her assistance in this work.

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Determination of Free Carbon in Butyl Reclaim by Modified A.S.T.M. Method

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THE production and use of regenerated GR-I (Butyl) elastomer, which has scrap inner tubes as a main raw material, made it desirable to develop methods for analysis of the constituent materials. In applying the A.S.T.M. procedure (*1*), it was difficult to obtain satisfactory filtering and washing of the dissolved sample in determination of the free-carbon content, because of the inherent resistance of GR-I to nitric acid oxidation. In view of this factor, a satisfactory way to disintegrate GR-I reclaim was sought that would result in acceptable filtering and washing.

In fairly extended trial disintegrations with possible solvents or reagents (acetone, petroleum ether, chlorosulfonic acid, mineral-seal oil, and others), mineral-seal oil gave the best filtering and washing and was tested for accuracy of results. It was found satisfactory and selected for regular use.

APPARATUS AND REAGENTS

Reagents needed are described in A.S.T.M. D-297-43T. The apparatus consisted of pieces normally found in a general analytical laboratory.

OUTLINE OF METHOD

Essentially, the proposed method follows the A.S.T.M. method for free carbon content of rubber (*1*, Section 23), except that the *a* extraction with chloroform-acetone is omitted and a hot digestion in mineral-seal oil precedes the treatment with nitric acid.

The general steps in the analysis are, in order:

Preparation and weighing of sample
Digestion in hot mineral-seal oil for breakdown and partial separation of the elastomer fraction
Filtering
Alternate washing with solvents and hydrochloric acid to remove oils, waxes, and carbonates
Washing with nitric acid for further removal of mineral fillers, other than carbon, that would change upon ignition
Final washes with acetone–hydrochloric acid mix to remove last traces of oils or fillers
Drying, weighing, igniting, and final weighing to determine loss of carbon

PROCEDURE

Weigh a 0.5000-gram sample of the GR-I reclaim, previously sheeted thinly (approximately 0.5 mm. thick), and place in a 250-ml. Erlenmeyer flask. Add 50 ml. of mineral-seal oil, cover flask with a watch glass, and heat overnight (or 12 hours minimum) in an oven set at 150° C.

Remove from the oven and allow to cool to room temperature. Dilute the contents of the Erlenmeyer with about 200 ml. of petroleum ether and 50 ml. of benzene. Shake carefully, to mix solvents, then filter with moderate suction through a Gooch crucible fitted with a moderately heavy asbestos mat. In this first transferral to the Gooch, take care that the crucible is not filled over 3/4 full, to prevent creepage of the carbon over top edge. Wash and police flask free of adhering particles, using hot acetone for the cleansing. Wash the solids, now all on the Gooch mat, using alternate applications of warm 10% hydrochloric acid and boiling acetone. Continue these alternate washings several times, until the filtrate comes through clear, washing last with 10% hydrochloric acid to avoid any violent reaction between acetone and the nitric acid added in next step. Empty the filter flask at this time to eliminate the same possible effect.

Resume treatment of the material in the Gooch, still using moderate suction, by washing once with cold nitric acid, five times with progressively warmer nitric acid, and three or four times with boiling nitric acid. After the nitric acid wash, empty the filter flask and resume washing, making the first filling of the Gooch with a mixture of equal parts (by volume) of acetone-chloroform–glacial acetic acid, followed by several washes with the same mixture, moderately heated. Next, wash with a hot 1 to 1 mix of acetone-chloroform and repeat until the filtrate runs clear, plus several additional washings. Finally, wash alternately with warm 10% hydrochloric acid and with boiling acetone until the filtrate is again clear. Remove the Gooch from the holder, clean the outside with a cloth moistened in acetone, and dry in oven for 2.5 to 3 hours at 105° C. Cool in desiccator, weigh (*a*), and then burn off carbon in an electric muffle at 800° C. Cool in desiccator and weigh again (*b*). The difference in weight ($a - b$) represents approximately 105% of the carbon originally present in the form of lampblack or gas black.

CALCULATION.

$$\% \text{ free carbon} = \frac{a - b}{1.05 \times \text{weight of sample}} \times 100$$

a = weight of crucible with carbon
b = weight of crucible after ignition
1.05 = factor covering effect of nitric acid on carbon black

DATA

In making determinations on Butyl (GR-I) reclaims, three samples of different production lots were run, with three parallel tests on each sample. Results are shown in Table I.

Such values indicate satisfactorily close agreement between check or parallel tests on a given sample, with a maximum deviation from the average of 0.19% in a given series. When comparing results from different samples, a somewhat greater deviation is noted, but this is undoubtedly due to existing differences in composition found from one batch to another, resulting from the fact that varying amounts of reclaiming oils are lost in processing.

While not of direct connection to this work, this modified procedure was used in determining the free carbon content of a cured, unreclaimed Butyl (GR-I) tube stock. Such trial indicated that the method is usable and fairly accurate, but further cheeking is desirable before applying it to the vulcanized stock. Data on vulcanized Butyl stock are given in Table II.

Table I. Carbon in Butyl Reclaims

Sample	Per Cent Free Carbon Test 1	Test 2	Test 3	Av.	Maximum Deviation from Average
A	30.86	31.07	31.00	30.98	0.12
B	31.16	31.39	31.27	31.27	0.12
C	30.09	30.22	29.81	30.04	0.19
Av.	30.70	30.89	30.69	...	...
Maximum deviation from average	0.61	0.67	0.88		
Theoretical				31.00	

Table II. Per Cent of Free Carbon in Vulcanized Butyl Stock

Sample	Test 1	Test 2	Av.
D	31.50	32.19	31.85
E	31.81	31.60	31.71

Calculated value = 30.30

SUMMARY

Because of difficulty in filtering and washing when the regular A.S.T.M. method for free carbon was applied to Butyl reclaim, a modified procedure was developed. The essential change is the addition of a hot digestion in mineral-seal oil, rather than use of nitric acid alone for disintegration of the elastomer, with some variation in the solvent and acid washes used to eliminate fillers and oils. Improved speed and ease of manipulation were obtained, with results checking well, when compared with each other and with calculated values. While intended for use on reclaimed stock, preliminary trial on cured Butyl stock was satisfactory, from both technique and accuracy phases.

ACKNOWLEDGMENT

The authors wish to thank Harry E. Rutledge, Goodyear Tire and Rubber Co. analytical laboratories, for valued consultation and scanning of the completed paper.

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A General Method of Color Grading

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A general method of color grading, based on the I.C.I. system, is described. This method is designed for products in which the colors of all samples of the given product follow a known relationship in color space; only a single number is needed to specify the color of a sample. An example is given of the setting up of a color-grading method for liquid terpene hydrocarbons based on the general principles discussed in the paper. An empirical expression for the locus of chromaticities of a number of representative samples from plant production was first established. Next, the chromaticities of hypothetical standards were selected such that each tenth-grade represented approximately four just perceptible differences. Thirdly, actual physical standards composed of mixtures of color-stable inorganic salts in aqueous solutions were prepared to match as closely as possible the color characteristics of the selected hypothetical standards. Suggestions are given for the use of these physical standards in connection with a photoelectric photometer for grading unknown samples. The use of similar color scales for cellulose plastics and cotton linters is also cited. It is often possible to grade products on the basis of existing color scales. An example is given of the application of the well-known U. S. rosin standards and Gardner standards to the grading of Thanite, a liquid insecticide ingredient.

WHENEVER industry has been confronted with the necessity of providing color specifications of a product, one of two courses has usually been followed: (*a*) an attempt has been made to use an existing color system, or (*b*) a new one has been invented. As a result of (*a*), many materials are now being color-graded on the basis of standards which have color characteristics only approximating those of samples of the corresponding grades. As a result of (*b*), a multiplicity of color-grading systems bearing little or no relationship to one another has come into more or less general use (*4*). Such procedures have some justification from the standpoint of expediency, but they are very apt to increase the confusion which surrounds the already complex subject of color.

Most product color specifications are based on a single-letter or single-number system—e.g., U. S. official rosin standards, Gardner standards, etc. Only those products having color characteristics similar to those of the standards of a given system may be graded by that system. Other specifications are based on a three-number (trichromatic) system—e.g., the Lovibond and Munsell systems. At present, the three-number system most widely recognized is the I.C.I. tristimulus system of color specification (*7, 13*). Practically all classes of products may be graded on the basis of a trichromatic system. However, a three-number specification is inconvenient to obtain and hard to interpret.

It would appear to be a hopeless task to set up a universal single-number color-grading system applicable to all types of colored materials. However, the first step toward alleviation of the confusion attending the large number of existing single-number color scales might very well consist of reducing these scales to a common denominator—i.e., converting them to the universal I.C.I. tristimulus system. A good start has already been made in this direction (*1, 3, 5, 6, 10, 12*). Furthermore, whenever it is necessary to set up a new scale for a given product or class of products, this scale too should be given in terms of I.C.I. data. The method by means of which such a scale can be set up is the subject of this paper. It is presumed in what follows that the reader is familiar with the terminology of the I.C.I. system (*7, 13*).

In order to establish such color scales, one must have access to a means for determining the I.C.I. specifications of the materials under consideration. These can be conveniently calculated from spectral transmittance or reflectance curves.

For the purpose of this discussion, the authors wish to draw a distinction between "color specification" and "color grading". By the term "color specification" is meant the designation of a point in the color solid which corresponds to a plot of the x- and y-trichromatic coefficients and the luminous transmittance or reflectance (Y-tristimulus value) of the sample in question. By "color grading" is meant the designation of the position of a point relative to a scale of grade numbers or letters on a preestablished two-dimensional chromaticity curve or a three-dimensional color (chromaticity-Y) curve. Such a curve is the locus of points representing the colors of a large number of representative samples of the given product for which the grading method has been devised. The following discussion will make this distinction more clear.

Suppose, for example, that we wish to determine the color of a given sample of a certain product. One method might consist of determining three numbers—e.g., I.C.I. X-, Y-, and Z-tristimulus values, or x-, and y-trichromatic coefficients and Y—with respect to some illuminant. Any one set of three such numbers will completely specify the appearance of a uniform sam le for any one particular illuminating and viewing condition. However, it is difficult for the average person to visualize a color from such a three-number specification. A specification consisting of but a single number would be much more practical. Such a single-number specification could be used if the colors of all samples of a given product followed a known relationship in color space.

For example, let us assume that the colors of samples of the product we are investigating obey a relationship such as that represented by the space curve AB of Figure 1. Let us further assume that we have arbitrarily set up a sequence of numerical grades (from 0 to 8 on Figure 1) based on equal increments of x-trichromatic coefficient (Δx). Then, a determination of any one of the three coordinates of a sample color determines uniquely a point on the space curve. The position of this point, as read off the numerical grading scale, is taken as the single-number specification which we seek.

Such a procedure of determining a single-number color specification is hereafter referred to as color grading, as opposed to three-number color specification. Color grading, as defined above, can be accomplished only when the plotted product colors lie on, or reasonably close to, an average curve such as AB. If

the product colors are widely scattered—e.g., if some samples are of various shades of blue, others green, and still others red—samples cannot ordinarily be graded on a single scale; it is usually necessary to determine a three-number specification for each sample.

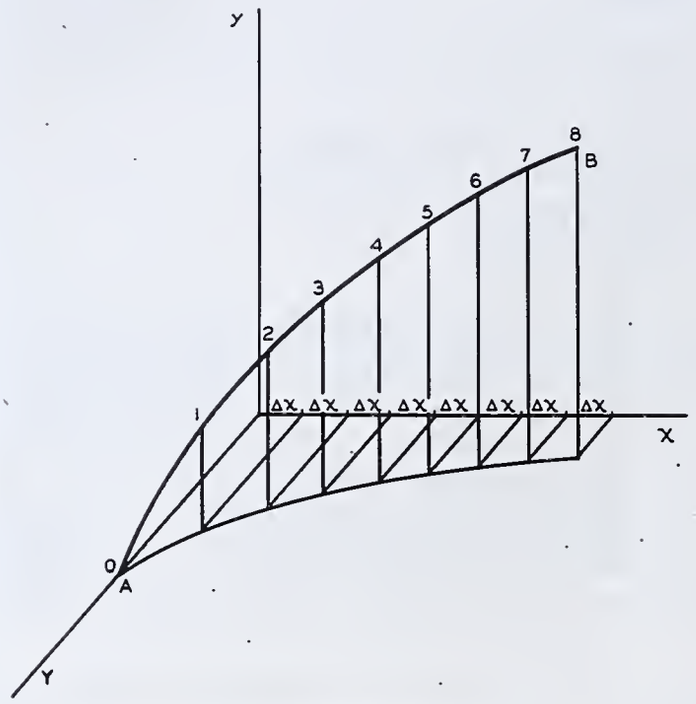

Figure 1. Three-Dimensional Product Locus

It has been found by repeated experiments in this laboratory that the eye has a tendency to neglect reasonably large differences in luminous transmittance during the visual color grading of transparent materials. This is fortunate, because, in some cases, as in the grading of gum rosin, rather large variations in this property occur in samples having the same chromaticities. While some tendency to downgrade samples of low Y value has been observed, this tendency is not so marked as one might expect. In the color grading of refined products, such as those dealt with in this paper, only small differences in Y among samples of the same chromaticity exist. Hence, methods of grading have been developed with the aid of a simple two-dimensional chromaticity diagram (x versus y) instead of a three-dimensional plot such as that of Figure 1. Frequently, however, variations in luminous transmittance or reflectance among samples of the same chromaticity are too large to neglect. In such cases, a notation giving information regarding Y should accompany each single-number grading.

TYPICAL COLOR SCALE

In order to illustrate the foregoing principles, a description will be given of the development of a method of grading a series of liquid terpene hydrocarbons, all of which had been refined by fractional distillation, and samples of which varied in color from practically water-white to dark amber. The first step in the procedure consisted of obtaining a large number of samples from regular plant production. Spectral transmission curves were obtained on these samples, and from the curves the x- and y-trichromatic coefficients and luminous transmittances with respect to Illuminant C were calculated. Representative curves from the set are shown in Figure 2, and trichromatic coefficients and luminous transmittances are given in Table I for the entire set. Figure 3 is a plot of x versus y from the data of Table I. Although there is some spread of the points, they follow a definite locus on the chromaticity diagram.

Table I. x- and y-Trichromatic Coefficients and Luminous Transmittances of Samples of Liquid Terpene Hydrocarbons Refined by Fractional Distillation

(50-mm. cell thickness)

Sample No.	x	y	Luminous Transmittance, Y, %
Illuminant C	0.3101	0.3163	92
1	0.3110	0.3190	90.40
2	0.3111	0.3184	90.67
3	0.3112	0.3197	90.74
4	0.3120	0.3201	90.22
5	0.3128	0.3211	89.21
6	0.3131	0.3222	90.74
7	0.3134	0.3229	90.29
8	0.3135	0.3221	89.37
9	0.3146	0.3246	89.72
10	0.3148	0.3254	90.72
11	0.3155	0.3275	90.34
12	0.3156	0.3268	90.09
13	0.3174	0.3296	89.19
14	0.3185	0.3305	88.04
15	0.3185	0.3310	88.90
16	0.3198	0.3321	88.06
17	0.3206	0.3342	89.67
18	0.3215	0.3359	87.75
19	0.3226	0.3389	89.56
20	0.3239	0.3396	89.07
21	0.3253	0.3400	86.26
22	0.3287	0.3482	88.73
23	0.3299	0.3505	87.90
24	0.3415	0.3678	86.53
25	0.3416	0.3694	86.40
26	0.3487	0.3801	85.50
27	0.3876	0.4190	72.99
28	0.3948	0.4265	74.79
29	0.4487	0.4722	64.09

In order to aid in establishing the position of this locus, it is often helpful, although not always necessary, to rectify the plot—i.e., to select coordinates which will yield a straight line. This has been done in Figure 4 which was plotted from the data of Table II. After coordinates have been selected such that the data plot out in a close approximation to a straight line, the usual methods of establishing the equation of the curve may be applied. In the present case, the equation was found to be

$$y = 0.3701 \log 100 (x - 0.2236) - 0.0267 \quad (1)$$

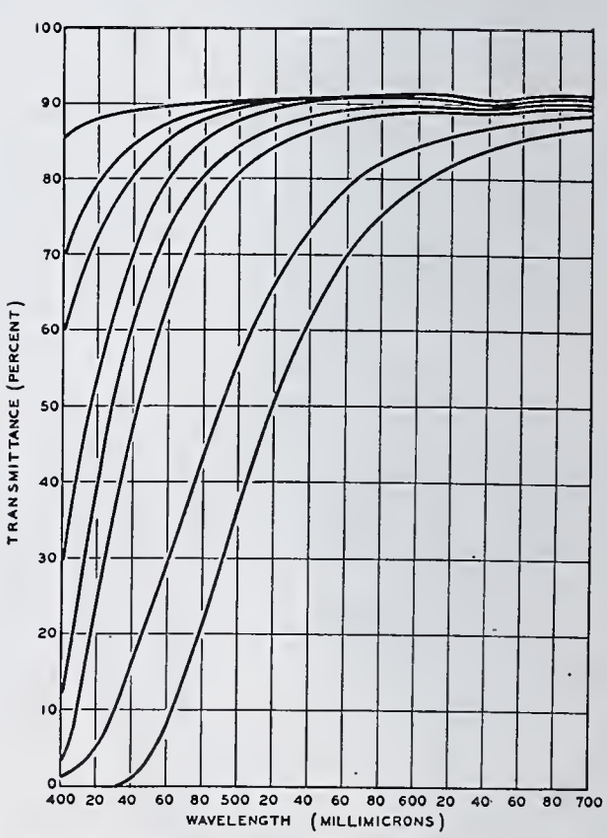

Figure 2. Spectral Transmittance Curves of Typical Terpene Hydrocarbons

Cell thickness, 50 mm.

y the method of averages. General methods of calculating mpirical equations may be found in several references (*2*, *11*).

If enough samples are measured to ensure a representative ross section of plant production, and if the manufacturing rocess is held constant, this equation may be considered as the efining equation of the locus of points representing the colors of amples of the product. However, if at any time the manuacturing process is modified in such a way as to change the haracter of the color of the samples, a new set of colorimetric ata should be obtained, and a new equation calculated.

Once the basic defining equation is established, the next step onsists of setting up standards in terms of which routine grading esults may be expressed. The spacing of these standards is an ntirely arbitrary procedure and depends on the accuracy with hich it is desired to grade. Generally, there is no point in esablishing fractional grade differences which are less than minium perceptible differences. Whether or not they are less can e determined by replotting the locus on a uniform chromaticity iagram such as that proposed by Judd (*8*, *9*). Often, however, isual examination of a number of specimens which have small erceptible color differences, and whose chromaticities are known,

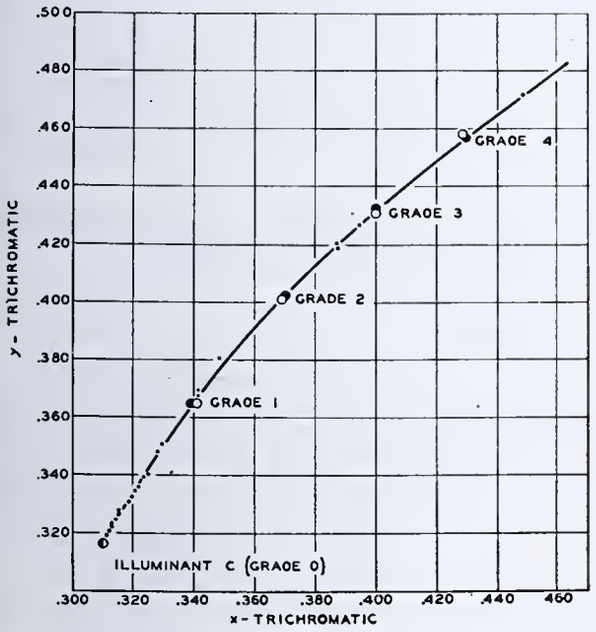

Figure 3. Chromaticities of Terpene Hydrocarbons

• Terpene hydrocarbons
● Arbitrarily chosen hypothetical standards
○ Actual working standards

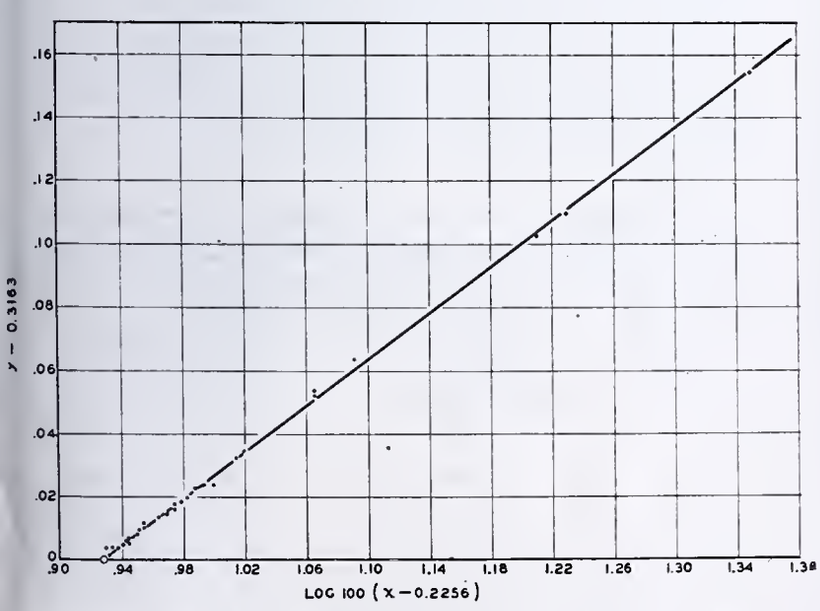

Figure 4. Rectified Plot of Chromaticities of Terpene Hydrocarbons

Table II. Data for Rectified Plot of Chromaticities of Terpene Hydrocarbons

Sample No.	x	$x - 0.2256$	log 100 × $(x - 0.2256)$	y	$y - 0.3163$
Illuminant C	0.3101	0.0845	0.9269	0.3163	0
1	0.3110	0.0854	0.9315	0.3190	0.0027
2	0.3111	0.0855	0.9320	0.3184	0.0021
3	0.3112	0.0856	0.9325	0.3197	0.0034
4	0.3120	0.0864	0.9365	0.3201	0.0038
5	0.3128	0.0872	0.9405	0.3211	0.0048
6	0.3131	0.0875	0.9420	0.3222	0.0059
7	0.3134	0.0878	0.9435	0.3229	0.0066
8	0.3135	0.0879	0.9440	0.3221	0.0058
9	0.3146	0.0890	0.9494	0.3246	0.0083
10	0.3148	0.0892	0.9504	0.3254	0.0091
11	0.3155	0.0899	0.9538	0.3275	0.0112
12	0.3156	0.0900	0.9542	0.3268	0.0105
13	0.3174	0.0918	0.9628	0.3296	0.0133
14	0.3185	0.0929	0.9680	0.3305	0.0142
15	0.3185	0.0929	0.9680	0.3310	0.0147
16	0.3198	0.0942	0.9740	0.3321	0.0158
17	0.3206	0.0950	0.9777	0.3342	0.0179
18	0.3215	0.0959	0.9818	0.3359	0.0196
19	0.3226	0.0970	0.9868	0.3389	0.0226
20	0.3239	0.0983	0.9926	0.3396	0.0233
21	0.3253	0.0997	0.9987	0.3400	0.0237
22	0.3287	0.1031	1.0133	0.3482	0.0319
23	0.3299	0.1043	1.0183	0.3505	0.0342
24	0.3415	0.1159	1.0641	0.3678	0.0515
25	0.3416	0.1160	1.0645	0.3694	0.0531
26	0.3487	0.1231	1.0903	0.3801	0.0638
27	0.3876	0.1620	1.2095	0.4190	0.1027
28	0.3948	0.1692	1.2284	0.4265	0.1102
29	0.4487	0.2231	1.3485	0.4722	0.1559

will be sufficient for the selection of a practical unit of fractional grading.

For example, a number of the samples represented on the chromaticity diagram of Figure 3 were examined visually, and it was decided that two samples having a difference in x-trichromatic coefficients of approximately 0.0030 could easily be distinguished under ordinary north sky illumination. This difference was called one-tenth grade. A Δx of 0.0300, therefore, represents one full grade. Actually it was found, by reference to the Judd uniform chromaticity diagram, that one-tenth grade represents, on the average, approximately four just perceptible differences. However, for our purpose, the grading unit was considered sufficiently small.

The grades increase in numerical value from 0, which represents a water-white sample—i.e., one having a chromaticity equal to that of Illuminant C—to 4, which represents a standard about as dark as any sample encountered in the representative lot. In Figure 3 are shown the positions of these arbitrarily chosen standards on the product locus.

The spacing of standards may be done on the basis of one of the other colorimetric quantities instead of x. Thus, certain materials might better be graded on the basis of equal increments of y, still others on the basis of equal increments of z, excitation purity, or dominant wave length. An even better method of spacing would be based on equal distances on one of the uniform chromaticity diagrams cited above, since equal spacings on these diagrams more closely approximate corresponding estimated visual differences than equal spacings on the I.C.I. diagram.

Through the medium of such data interlaboratory comparisons of color scales could readily be made. Color scales "tailor-made" to fit the products for which they were intended could be set up without fear that the resulting large number of scales would introduce unwarranted complexity. Correspondence between laboratories involving color scale comparisons would necessarily include tables of x, y, and Y values of numbered standards on the scales under discussion. Alternatively, one might use equations such as Equation 1 or plots such as that in Figure 3 for such comparisons.

STANDARDS

The next step in establishing the grading method consists of devising actual physical standards, the chroma-

ticities of which will match as nearly as possible the chromaticities of the arbitrarily chosen hypothetical standards. Physical standards may consist of: (1) specimens of the material itself which are chosen to match in color the hypothetical standards; (2) standards consisting of combinations of colored glasses; or (3) standards composed of mixtures of solutions of organic or inorganic compounds. Any set of standards chosen should have the following characteristics: (1) The spectral transmittance curves should be similar in character to those of the material being graded. (2) The standards should have reasonably permanent color characteristics over a long period of time. (3) Their colors should not be appreciably altered by variations in temperature over the range from 15° to 30° C. (4) They should be easy to prepare.

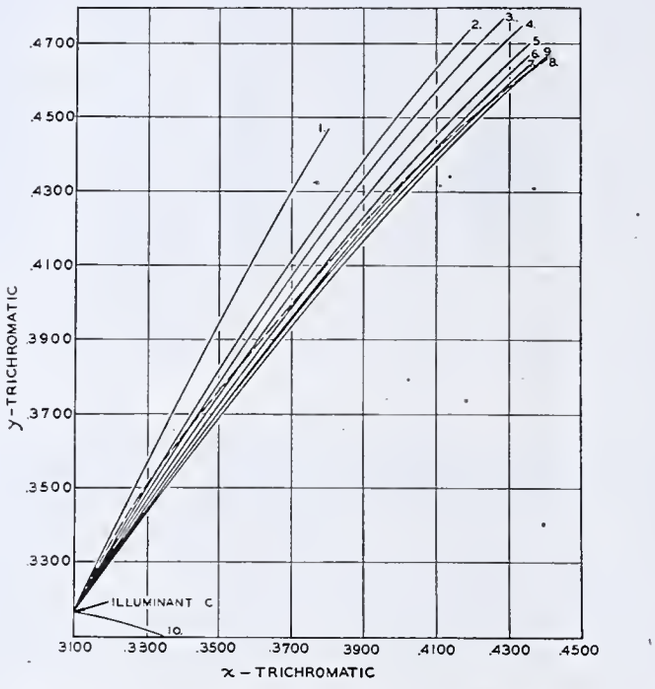

Figure 5. Chromaticity Loci of Acid Aqueous Solutions of $UO_2(NO_3)_2.6H_2O$, K_2PtCl_6, and $CoCl_2.6H_2O$

1. Various thicknesses of $UO_2(NO_3)_2.6H_2O$ stock solution
2. Various thicknesses of 50% $UO_2(NO_3)_2.6H_2O$ stock solution + 50% K_2PtCl_6 stock solution
3. Various thicknesses of 35% $UO_2(NO_3)_2.6H_2O$ stock solution + 65% K_2PtCl_6 stock solution
4. Various thicknesses of 20% $UO_2(NO_3)_2.6H_2O$ stock solution + 80% K_2PtCl_6 stock solution
5. Various thicknesses of 10% $UO_2(NO_3)_2.6H_2O$ stock solution + 90% K_2PtCl_6 stock solution
6. Various thicknesses of K_2PtCl_6 stock solution
7. Various thicknesses of 94% K_2PtCl_6 stock solution + 6% $CoCl_2.6H_2O$ stock solution
8. Hydrocarbon product locus
9. Various thickness of 92% K_2PtCl_6 stock solution + 8% $CoCl_2.6H_2O$ stock solution
10. Various thicknesses of $CoCl_2.6H_2O$ stock solution

Standards prepared from the material itself usually have most of the desirable characteristics enumerated above, except that of permanence. This characteristic may often be improved by sealing the standards in air-tight glass containers under vacuum or in inert atmospheres, and by storing them in the dark. When using such standards, their spectral transmittances should be checked frequently to determine whether any appreciable change in color with time is taking place. If small changes do occur, the nominal value of each of the standards may be adjusted from time to time to compensate for such changes.

Although glass standards are usually satisfactory, it may be found that because of the limited number of suitable types of glass available, it may be impossible to match the hypothetical standards accurately. Moreover, the calculations involved in determining the relative thicknesses of two or more components necessary to match a given hypothetical standard must be repeated each time it is necessary to replace the standards, unless the glasses for the replacement set are taken from the same melts as used previously. The glass elements must be accurately ground to the calculated thicknesses to avoid error. This means that considerable expense is involved in making up a set.

On the other hand, many liquid standards, especially those made with acid solutions of certain inorganic salts, have excellent color stability and have the great advantage of being easy to prepare. It may be difficult to choose a liquid or combination of liquids having spectral transmittance curves similar to those of the material being color graded. However, in many cases, a sufficiently good approximation may be obtained for practical grading.

In the present example, a series of liquid standards was prepared from acid solutions of the following salts which are known to be color-stable: uranyl nitrate hexahydrate, potassium chloroplatinate, and cobalt chloride hexahydrate. The reason for this choice will be evident from an examination of Figure 5, which shows the loci of points representing the colors of different thicknesses of stock solutions of each of the three salts and of various mixtures of stock solutions of these salts on a chromaticity diagram, together with the product locus of Figure 3.

The stock solution of potassium chloroplatinate was prepared with 1 gram of potassium chloroplatinate plus 50 cc. of concentrated hydrochloric acid made up to a volume of 500 cc. with distilled water. The stock solution of cobalt chloride hexahydrate was prepared with 1 gram of cobalt chloride hexahydrate plus 50 cc. of concentrated hydrochloric acid made up to a volume of 500 cc. with distilled water. The stock solution of uranyl nitrate hexahydrate was prepared with 2 grams of uranyl nitrate hexahydrate made up to a volume of 50 cc. with distilled water.

Since these solutions were known to obey Beer's law, different thicknesses instead of different concentrations were used for convenience. Hence, in order to obtain any given locus, spectral transmittance curves of only one solution in matched optical cells of different thicknesses were run.

In Figure 5, it will be seen that the potassium chloroplatinate locus (curve 6) crosses the product locus (curve 8) at a point near the upper end of the diagram. The locus of points representing different thicknesses of a 90%-10% mixture of stock solutions of the two salts, potassium chloroplatinate and uranyl nitrate, respectively (curve 5), crosses the product locus at a point nearer the illuminant point. An 80%-20% mixture yields a locus (curve 4) which crosses at a point still nearer, and so on. A plot is then made of the x-trichromatic coefficients of the crossing points versus the concentration of one of the constituents, as shown in Figure 6, *a*.

In the case of standards darker than those represented by the crossing point of the potassium chloroplatinate stock solution locus, mixtures of potassium chloroplatinate and cobalt chloride stock solutions were used to give crossing points on the upper part of the product locus. Figure 6, *b*, is a plot of these crossing points versus the concentration of cobalt chloride stock solution. From these curves the desired relative concentrations of the stock solutions which will yield a locus crossing the product locus at a point representing the chromaticity of a hypothetical standard may be found. Table III shows the relative concentrations of stock solutions of the salts necessary to yield such loci. These data were obtained directly from Figure 6.

Table III. Relative Concentrations of Stock Solutions Necessary to Yield Loci Which Cross Product Locus at Chromaticities Equal to Those of Selected Standards

Standard No.	x-Trichromatic Coefficient	Relative Concentrations of Stock Solutions
0	0.3100 (actually 0.3101)	Distilled H_2O
1	0.3400	38.0% $UO_2(NO_3)_2.6H_2O$ + 62.0% K_2PtCl_6
2	0.3700	11.0% $UO_2(NO_3)_2.6H_2O$ + 89.0% K_2PtCl_6
3	0.4000	0.5% $UO_2(NO_3)_2.6H_2O$ + 99.5% K_2PtCl_6
4	0.4300	7.1% $CoCl_2.6H_2O$ + 92.9% K_2PtCl_6

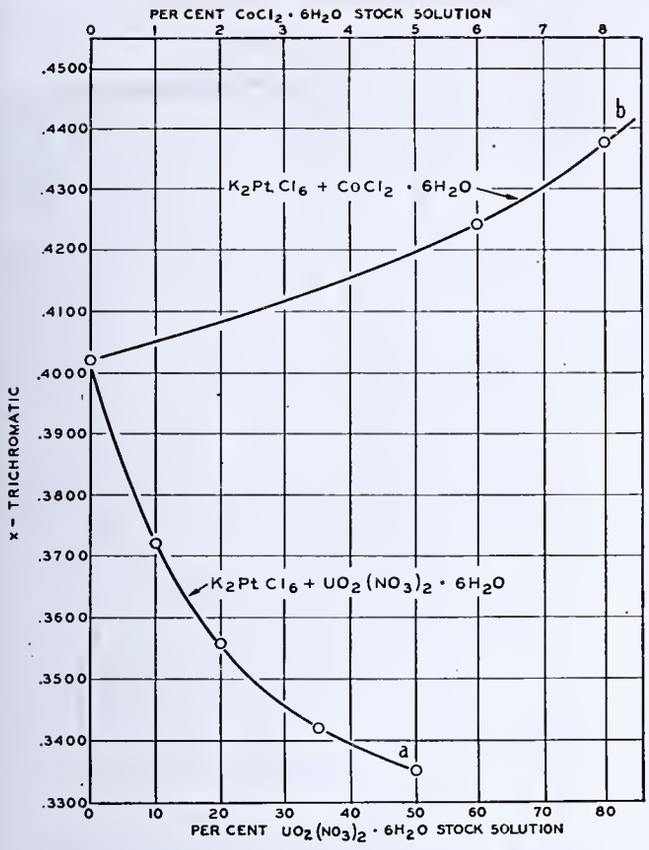

Figure 6. x-Trichromatic Coefficients of Crossing Points of Solution Loci and Product Locus Versus Relative Concentration of One Solution Constituent

Next, spectral transmittance curves of different thicknesses of solutions made up in accordance with Table III were run, and the corresponding chromaticities were computed. The x-trichromatic coefficients corresponding to the different thicknesses of each of these solutions are given in Table IV. The thicknesses, t, corresponding to x-trichromatic coefficients equal to those of the desired hypothetical standards may then be interpolated from the table or from a curve of t versus x.

Since Beer's law holds, solutions of thickness t are equivalent colorimetrically to 5 cm. of solution diluted in the ratio of t parts of original solution to $(5 - t)$ parts of distilled water. Using this relationship, the proper concentrations of the various constituents in 5 cm. of aqueous solution to yield each of the hypothetical standards were determined. These are recorded in Table V. The solutions were then made up in accordance with the compositions listed in Table V, placed in 5-cm. glass absorption cells, and sealed off.

As a final check, spectrophotometric data were obtained on each standard (Table V). The locus connecting these points on a chromaticity diagram should coincide with the product locus. In the actual case considered here, the agreement is shown in Figure 3.

The foregoing procedure represents a considerable expenditure of labor. However, once the standards have been contrived, they may be reproduced with ease at any time. The cells in which the standards were sealed can be made by an experienced glass blower for approximately $1.50 each. In this work, 5-cm. cells were used instead of cells of less thickness, since they are somewhat easier to hold within the required thickness tolerance, which is of the order of $\pm 1\%$.

FILTERS

The next step in setting up the grading method is to choose a pair of filters to be used on a suitable photoelectric photometer for obtaining quantities proportional to the established grades. Two filters or filter combinations, one of which is selected to yield a photocell response proportional to the X-tristimulus value, and the other of which is selected to yield a photocell response proportional to the sum of the X-, Y-, and Z-tristimulus values, will give, when the ratio of the first to the second is computed, a product proportional to the x-trichromatic coefficient (since $\frac{X}{X + Y + Z} = x$).

However, a pair of filters which would be more desirable from the standpoint of sensitivity and response could be selected from an examination of the spectrophotometric curves of samples of the product. It will be seen from an examination of Figure 2 that from sample to sample, the greatest change takes place in the blue and violet regions of the spectrum, while very little change takes place in the red region. Hence, for maximum sensitivity, one would assume that a ratio of blue to red filter readings would be the most satisfactory. However, most of the commercially available red glasses are of the selenium type. These have high temperature coefficients which make them unreliable for ordinary routine grading purposes. On the other hand, amber (orange) filters can be made up from glasses having negligible temperature coefficients. It will be seen from Figure 2 that while slightly greater changes occur in the orange than in the red region of the spectrum between different samples, the changes are still small compared to those occurring in the blue and violet regions. Amber and violet filters were used with a photoelectric photometer to obtain the calibration curve of Figure 7, in which instrument readings are plotted against the grades of the previously described set of five liquid standards.

Table IV. x vs. Thickness for Solutions Made Up in Accordance with Table III

Relative Concentrations of Stock Solutions	Thickness, Cm.	x-Trichromatic Coefficient
38.0% $UO_2(NO_3)_2.6H_2O$ + 62.0% K_2PtCl_6	0	0.3101
	1	0.3435
	1.8	0.3662
	3	0.3941
	5	0.4271
11.0% $UO_2(NO_3)_2.6H_2O$ + 89.0% K_2PtCl_6	0	0.3101
	1	0.3435
	1.8	0.3672
	3	0.3967
	5	0.4349
0.5% $UO_2(NO_3)_2.6H_2O$ + 99.5% K_2PtCl_6	0	0.3101
	1	0.3434
	1.8	0.3673
	3	0.3981
	5	0.4370
7.1% $CoCl_2.6H_2O$ + 92.9% K_2PtCl_6	0	0.3101
	1	0.3414
	1.8	0.3644
	3	0.3943
	5	0.4331

Table V. Calculated Concentrations of Salts in Aqueous Solutions and 5-Cm. Thicknesses to Color Match Hypothetical Standards

Standard No.	x	y	Composition
0[a]	0.3104	0.3182	Distilled H_2O
1	0.3385	0.3646	0.22 gram of K_2PtCl_6 + 2.64 gram of $UO_2(NO_3)_2.6H_2O$ + 10.8 cc. of HCl made up to 1 liter with distilled H_2O
2	0.3629	0.3952	0.67 gram of K_2PtCl_6 + 1.66 gram of $UO_2(NO_3)_2.6H_2O$ + 33.6 cc. of HCl made up to 1 liter with distilled H_2O
3	0.3984	0.4306	1.22 grams of K_2PtCl_6 + 0.12 gram of $UO_2(NO_3)_2.6H_2O$ + 61.1 cc. of HCl made up to 1 liter with distilled H_2O
4	0.4307	0.4589	1.79 grams of K_2PtCl_6 + 0.14 gram of $UO_2(NO_3)_2.6H_2O$ + 96.2 cc. of HCl made up to 1 liter with distilled H_2O

[a] Chromaticity differs from that of Illuminant C because of color of glass cell windows.

Since the grading method was set up on the basis of the I.C.I. system, the use of filters other than those yielding I.C.I. specifications will give rise to grading errors if the samples and standards are not spectrally similar. A method of correcting for such errors is discussed more fully below.

PHOTOELECTRIC PHOTOMETERS

The following basic design features in photoelectric photometers have been found desirable for trouble-free color grading:

An essentially parallel light beam of just sufficient intensity to give adequate sensitivity.

An absorption cell with plane glass windows which should be of reasonable optical quality essentially free from disturbing color in the glass.

A receiver consisting of one or more barrier-layer photocells, the faces of which are illuminated uniformly by the beam of light after it has passed through the sample; (these photocells should preferably be chosen to have high internal dark resistance and low fatigue).

A set of filters, the spectral transmittances of which preferably are so chosen with respect to the spectral sensitivity of the photocell and the spectral distribution of the light source that the photocell response (depending on the filter) is proportional to one of the tristimulus values or to the sum of the three tristimulus values of the sample being measured.

An electrical circuit which offers a low external resistance to the photocells. The circuit preferably should be of the null type—e.g., one in which the desired transmittance readings are obtained from a value of resistance read on a dial or slide wire, or from a calibrated flux-reducing mechanism in the optical path.

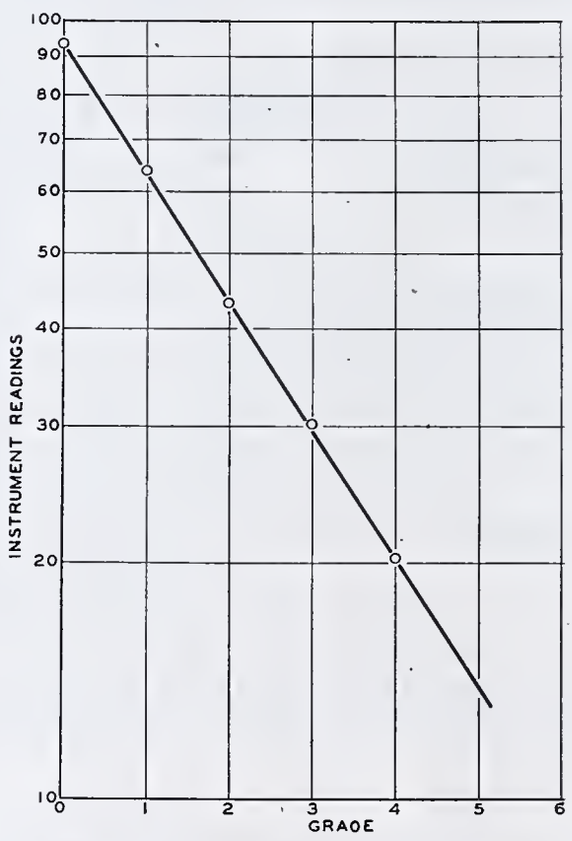

Figure 7. Photometer Calibration Curve

These fundamental characteristics of good design are found in several photometers now available on the market. In the work described in this report, a null-type photometer of the authors' own design was used. This instrument is so constructed that a ratio of one photocell response to another may be read directly from a slide wire.

CALIBRATION

Before unknown samples were graded, a calibration curve was prepared by inserting each of the standards in the light beam in turn, and taking the corresponding readings as described above. These readings were plotted against the actual grades of the standards on a sheet of single-cycle, semilogarithmic paper. It was fortuitous that a straight line resulted, as is shown in Figure 7. This circumstance makes it possible to plot a calibration curve with only two standards. In general, however, such a calibration would be a curve, and it would be necessary to run a complete set of standards each time a calibration was made. With some of the authors' instruments, this is done every few days and takes less than 5 minutes.

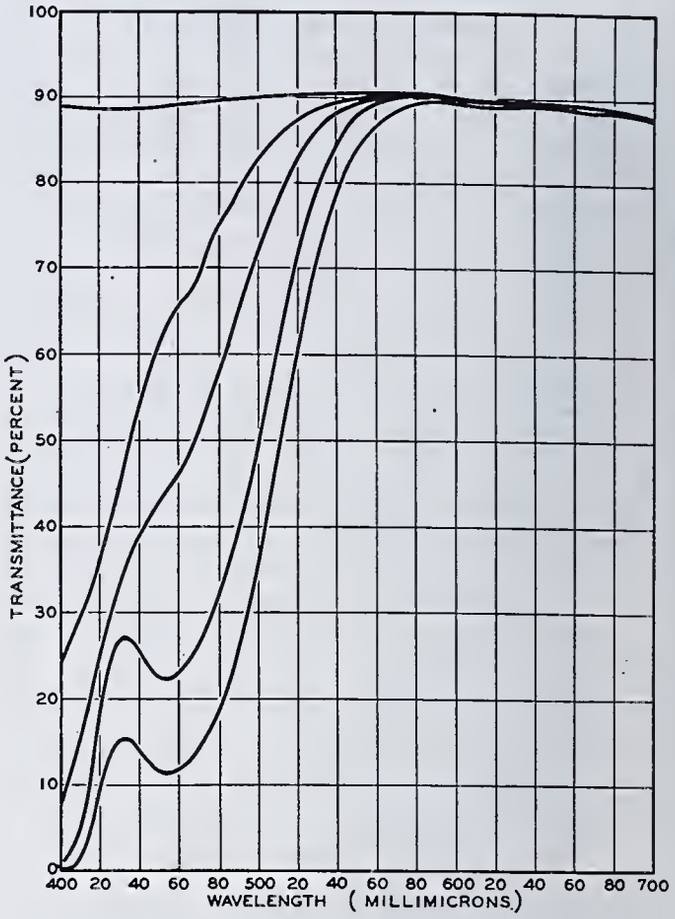

Figure 8. Spectral Transmittance Curves of Working Standards

Slight spectral selectivity of 0 standard due to small amount of color present in glass of cell windows

Samples were graded by pouring them in 5-cm. cells, inserting each of the cells in the light beam in turn, and taking instrument readings. Each reading was referred to the calibration curve, and the grade was read to the nearest tenth grade.

If samples and standards are not spectrally similar, a given sample having the same I.C.I. specifications as one of the standards will not necessarily grade the same as the standard on a photoelectric photometer using a pair of filters other than those carefully designed to yield I.C.I. specifications. However, the standards may still be used to check the calibration of the instrument periodically if the samples themselves are unstable. In such a case, the proper corrections may be computed by (1) calculating grades of a series of samples from I.C.I. data obtained spectrophotometrically and referred to the product locus, (2) calculating grades from photoelectric photometer readings referred to a calibration curve obtained for the photometer with the selected standards, and (3) computing differences in the grades obtained by the two methods. These differences may then be used to correct all readings subsequently made on the photometer.

This procedure will be clarified by describing its application to the grading of terpene hydrocarbons. The spectral dissimilarity between the samples and the inorganic standards may be seen by comparing the transmittance curves of the former (Figure 2)

with those of the latter (Figure 8). In order to establish a correction for this dissimilarity, the following procedure was used.

A number of samples selected from a group of those falling near the product locus of Figure 3 were assigned fractional grades by interpolating their spectrophotometrically determined x-trichromatic coefficients between those of the arbitrarily chosen standards listed in Table III. These grades are listed in column 4 of Table VI. Next, readings were obtained on the grading photometer, and were referred to the calibration curve of Figure 7. Fractional grades were again assigned on the basis of this calibration curve, and are listed in column 5 of Table VI. Differences in the grades assigned by the two methods are given in column 6. In the case considered here, these differences were small enough to be neglected. In general, however, they could be used to correct the original calibration curve.

OTHER COLOR SCALES

This same scheme has also been applied successfully to other classes of compounds. Figure 9 represents a number of samples of cellulose plastics plotted on an (x, y)-chromaticity diagram. In this case, the agreement of all samples with a single locus is not as good as in the first case. This is partly due to the fact that examples of five different classes of compounds are included in the figure. The agreement is still close enough, however, to allow the same grading scheme to be used for all. Deviations are considered less important than the difficulty in applying and interpreting a three-number (or even a two-number) specification.

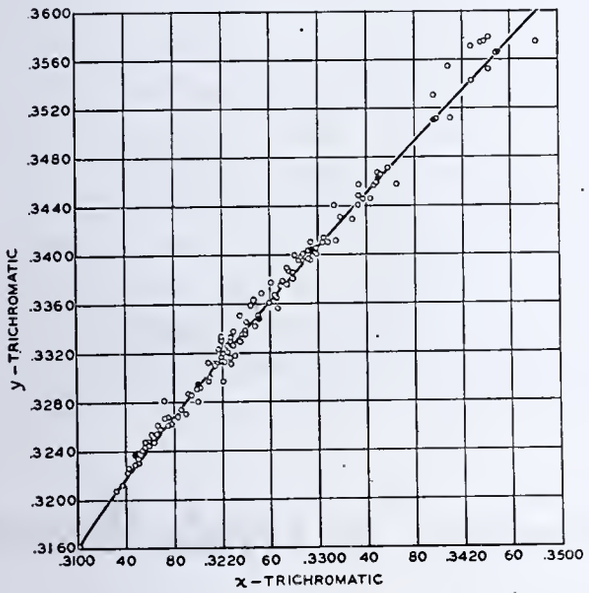

Figure 9. Chromaticities of Cellulose Products

○ Cellulose products samples
● Working standards

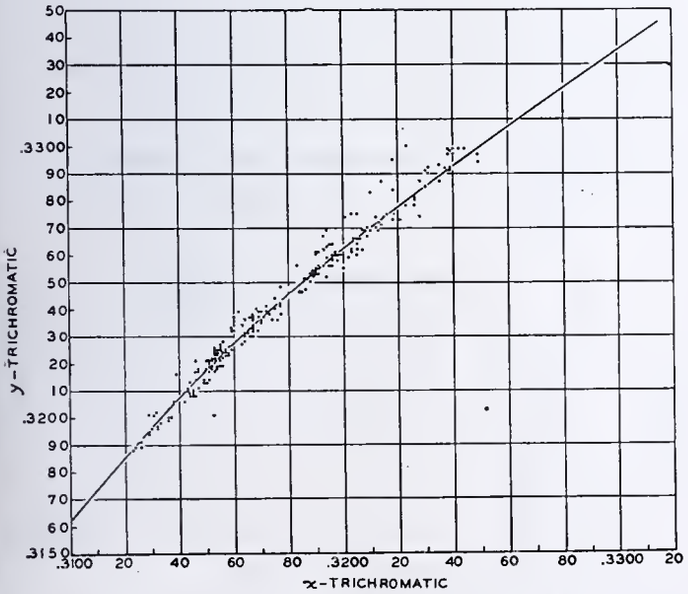

Figure 10. Chromaticities of Cotton Linters

Figure 10 is a product locus for cotton linters plotted from the chromaticities of a large number of pressed samples from regular plant production. About the same lack of agreement is evident here as in Figure 9.

Standards were chosen for both classes of products by means of the method described above, and were made of combinations of solutions of the same salts as used for the terpene standards.

Well-known color scales may often be applied without error to certain classes of products. For example, two types of standards whose use is widespread are the glass U. S. rosin standards sponsored by the United States Department of Agriculture (*1*), and the Gardner color standards used rather extensively in the varnish industry (*4*).

The chromaticity plots of a few standards of each of these two types are shown in Figure 11 in order to demonstrate the possibility of applying them to a color grading scheme for commercial grades of Thanite, a liquid insecticide ingredient. The U. S. rosin standards are nearly equally spaced with respect to their x-trichromatic coefficients. It can readily be seen that they may be used for grading the product in question. The Gardner standards down to No. 8 also fall on the locus of the product points, but their lack of equal or even regular spacing indicates that they should be modified to make them more useful in applying or interpreting a color-grading method based on them.

For rough grading these physical standards may often be used visually—e.g., in a Nessler tube. It would not, of course, be

Table VI. Corrections for Spectral Dissimilarity between Terpene Samples and Inorganic Standards

Designation	x	y	Grade Determined from I.C.I. Data	Grade Determined from Photometer Reading	ΔG
6a	0.3133	0.3223	0.11	0.09	−0.02
20a	0.3241	0.3388	0.47	0.51	0.04
23a	0.3351	0.3569	0.84	0.97	0.13
26a	0.3753	0.4110	2.18	2.31	0.13
28a	0.4241	0.4564	3.80	3.94	0.14

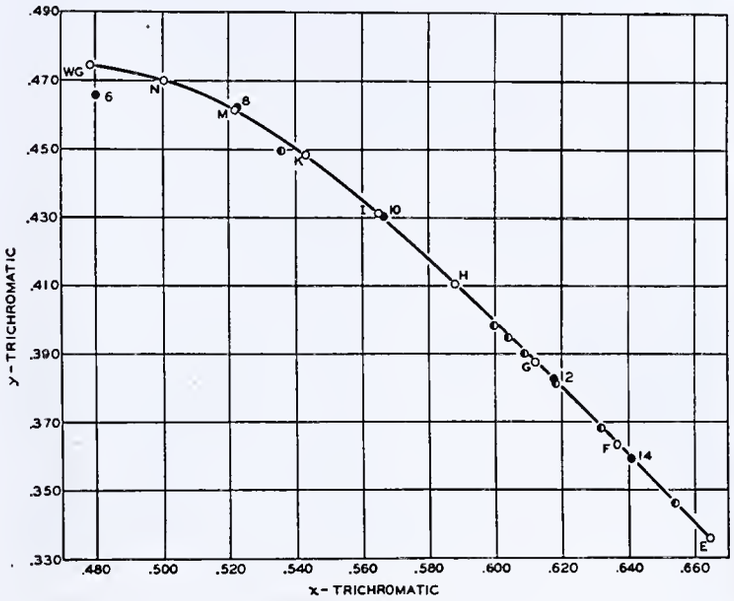

Figure 11. Chromaticities of Thanite Samples, U. S. Rosin Standards, and Gardner Standards

○ U. S. rosin standards
● Gardner standards
◐ Thanite samples
} Cell thickness, 50 mm.

valid to use variable thicknesses of a single standard in a Nessler tube unless it had been ascertained previously that Beer's law was obeyed by the mixtures comprising the standards. In the case of Gardner standards, varying the thickness does not correspond to changing the number. This is due to the fact that the standards are not merely dilutions of the darkest standard, but actually have different relative compositions. This condition also holds with the terpene standards discussed above.

SPECIAL CONSIDERATIONS

Very often, a product is encountered in which the chromaticity points of a large number of samples have rather wide dispersion—i.e., the scattering about the locus is large. In such a case, it may be necessary to set up tolerances. This may be done by drawing curves on either side of the product locus, enclosing all the points which, from experience, it is decided are "on-color". In the grading of such products, it will probably be necessary to determine both the x- and y-trichromatic coefficients of the sample by means of two suitable filter pairs. By reference to a curve in which a product locus with its tolerance band is drawn, it can be determined whether or not the color of the sample in question falls outside or inside the band. Thus, samples whose colors fall outside the band should be designated "off-color", and no attempt should be made to grade them on the foregoing basis. However, if it is desired to establish some sort of grading designation for the sample, it may, for example, be given a grade which corresponds to that of a sample lying on the curve of equivalent x-trichromatic, or better still, equivalent excitation purity, but with a notation to show that it is "off-color" low or "off-color" high, depending on whether the sample chromaticity lies below or above the tolerance band. Such a scheme may also be used to designate samples of low or high Y-value.

If the sample is known to be fluorescent, or if it is hazy, the color specification which can be most conveniently applied is that which one would normally make visually by means of viewing the sample with the aid of a directed beam of light passing through the sample toward the eye. This same result may be accomplished in a photoelectric instrument by excluding the scattered or fluorescent light from the receiver. This may easily be done by mounting the receiver at a considerable distance from the sample. Sometimes, however, it may be customary to grade samples of a certain product by viewing them with more or less indirect illumination. Such a case occurs when the grader lines up a series of samples on a sheet of white paper near a window. It will often be found that samples graded in this manner will not fall in the same order as if they were graded by direct light from the window, especially if the samples contain varying amounts of haze. Photoelectric grading, to correspond approximately with visual grading by indirect illumination, should be done with the sample very near the receiver. If an integrating sphere is used as the receiver, the sample may be mounted inside the sphere for such a measurement.

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Fluorometric Determination of Aluminum in Steels, Bronzes, and Minerals

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THE absence of specific sensitive reagents for the determination of aluminum is a notable handicap to the analytical chemist. This difficulty is reflected in the laborious procedures required for an accurate gravimetric analysis of small percentages of aluminum in steel (*1*, *6*, *12*) and in the fact that rock analysts usually calculate alumina by difference from the R_2O_3 precipitate (*5*).

The reagent which has found widest use is "aluminon", ammonium aurintricarboxylate (*4*, *8*, *15*). In spite of interference by chromium, iron, beryllium, vanadium, titanium, and gallium, it has been used for colorimetrically determining from 0.04 to 1.5% of aluminum in steel (*2*), and for low percentages in nonferrous alloys (*10*). Ether extraction and mercury cathode electrolysis were used, respectively, as separations in these two procedures.

But the need was still felt for a method, other than spectrographic (*11*), suitable for determining down to less than 0.01% of aluminum in such materials as steels, bronzes, and minerals. A quantitative fluorescence method for aluminum in pure solution using the reagent morin (*14*) was reported subject to several interferences, among them beryllium. However, the very sensitive qualitative reagent Pontachrome Blue Black R, a dye which gives a red fluorescence with aluminum (*13*), suffers from fewer interferences. A promising approach was thus offered, if the details could be worked out for a quantitative determination. The relative interferences in the fluorescence tests for aluminum by Blue Black R and by quercetin, an isomer of morin, have already been established (*3*).

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APPARATUS AND REAGENTS

Measurements were made with a Lumetron fluorescence meter, model 402EF, using the 25-ml. cell. The scale was set at 0 and 100 by means of fluorescing standards which contained known amounts of aluminum. Corning filter No. 5874 was used to isolate the ultraviolet energizing radiation, and sheets of red plastic to isolate the red fluorescent light emitted. The transmission of these filters is shown in Figure 1, as determined by a Beckman spectrophotometer.

Standard solution of aluminum, 1.00 ml. = 0.0100 mg. Dissolve 0.1760 gram of potassium aluminum sulfate crystals in water, and dilute to 1 liter.

Weaker standard solution of aluminum, 1.00 ml. = 0.00100 mg. Pipet out 100 ml. of the above solution, and dilute to 1 liter.

Ammonium acetate solution, 10%. Dissolve 50 grams of the salt in water and dilute to 500 ml.

Pontachrome Blue Black R (Color Index 202), 0.1% solution. This dye is the sodium or zinc salt of 4-sulfo-2-hydroxy-α-naphthalene-azo-β-naphthol. Dissolve 0.50 gram in 500 ml. of 95% ethyl alcohol, and allow to stand a few days before using. This dye may also be purchased under the name Superchrome Blue Black (National Aniline Division of Allied Chemical and Dye Corporation).

Pontachrome Violet SW (Color Index 169), 0.1% solution. Dissolve 0.50 gram in 500 ml. of 95% ethyl alcohol.

Sodium hydroxide solution, 10%. Dissolve 25 grams in 250 ml. of water in a large platinum dish, with the aid of a platinum stirring rod. Store in a ceresin bottle, to avoid aluminum contamination from glassware.

Dilute sulfuric acid, 1 to 9. Pipet 100 ml. of concentrated acid into several hundred milliliters of water in a 1000-ml. volumetric flask, cool, and dilute to the mark.

A rapid fluorometric method is described for the quantitative determination of from 0.001% to somewhat over 1% of aluminum in steels, bronzes, and minerals. This represents a range of 0.0002 to 0.025 mg. of aluminum in a volume of 50 ml. The necessity for special microtechnique is avoided by the use of aliquots of a macrosample; the sensitivity is better than 1 part in 100,000,000. For small percentages of aluminum, this method surpasses other procedures in speed, sensitivity, accuracy, and freedom from interference. The preferred reagent is the dyestuff Pontachrome Blue Black R, which is used at a pH of 4.8 in buffered solution. Excellent results may also be obtained with Pontachrome Violet SW. Electrolysis in a mercury cathode cell serves to eliminate interfering ions. Studies have been conducted on the changes in intensity of fluorescence under variation of experimental conditions such as temperature, time of standing, pH, dye concentration, and aluminum concentration. The probable composition of the fluorescent substance has been established by analysis, after amyl alcohol extraction from aqueous solution.

EXPERIMENTAL

It was necessary first to study the effect on the fluorescence intensity of such variables as pH, amount of dye, amount of aluminum, time of standing, and temperature.

Effect of pH. A series of twenty solutions was prepared in 50-ml. volumetric flasks. To each flask were added 0.050 mg. of aluminum, 5.0 ml. of 10% ammonium acetate as a buffer, 1.0 ml. of 0.1% Blue Black R dye, and quantities of acetic acid or ammonium hydroxide sufficient to give a range of pH from 2.4 to 9.8. The solutions were diluted to the mark, shaken vigorously, and allowed to stand for 2 hours before fluorescence measurements were taken. The pH was measured with the laboratory model Beckman pH meter. Under these conditions, the optimum pH is about 4.8 or 4.9 (Figure 2).

Repetition at the lower level of 0.010 mg. of aluminum gave similar results, except that the peak was considerably narrower.

Concentration of Blue Black R. A series of 15 solutions of 50-ml. volume was made up, each containing 0.050 mg. of aluminum, 0.50 gram of ammonium acetate, 0.10 ml. of glacial acetic acid to give a pH close to the optimum, and amounts of 0.1% dye solution varying from none to 5.0 ml. The solutions were allowed to stand 80 minutes before readings were taken. It is evident from Figure 3 that maximum intensity is achieved by the use of 1.5 ml. Solutions containing more than this amount showed a rapid decrease in fluorescence in those portions furthest from the ultraviolet source, indicating a progressive absorption of the energizing radiation.

Time of Standing. Since it was known that the fluorescence did not attain its maximum intensity immediately after mixing, the change of intensity with time was investigated. A solution was prepared which contained 0.010 mg. of aluminum, 0.50 gram of ammonium acetate, 1.0 ml. of 1 to 24 sulfuric acid, and 1.5 ml. of 0.1% Blue Black R in a volume of 50 ml. The temperature was 24° C. Measurements were made over a period of 1 hour, and another reading was taken after 3 hours. Figure 4 shows that equilibrium was attained in less than an hour. Repetition of the experiment at the higher level of 0.100 mg. of aluminum gave a similar result.

Temperature. In order to determine whether maximum intensity could be obtained in a shorter time by heating the solution, a solution containing 0.010 mg. of aluminum, 0.5 gram of ammonium acetate, and 0.05 ml. of acetic acid was diluted to 48 ml. and heated to 70° C., and then 1.5 ml. of dye solution were added. Readings of fluorescence were taken during the next 1.5 hours, as the solution cooled gradually down to room temperature. The intensity increases more slowly than that of a solution mixed at room temperature (Figure 5). Repetition at the level of 0.020 mg. of aluminum gave a similar result. The instrument scale was reset arbitrarily for each run, so that readings are not necessarily transferable from one graph to another.

The effect of cooling was studied next. A 50-ml. solution con-

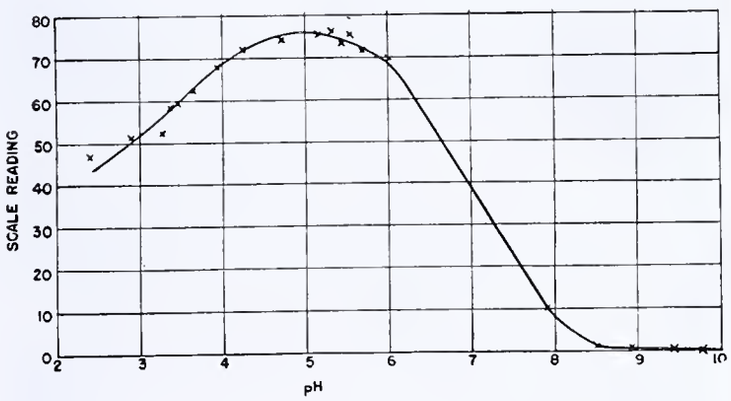

Figure 2. Effect of pH on Fluorescence Intensity

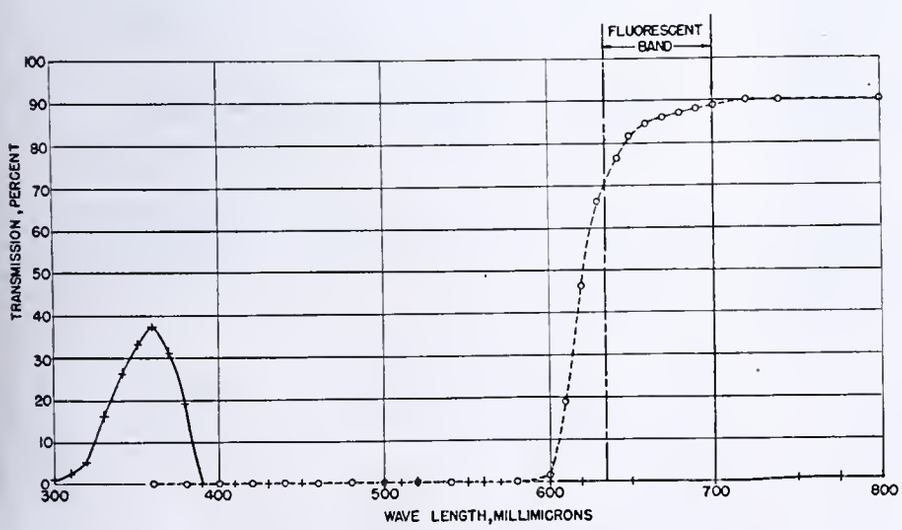

Figure 1. Transmission of Primary and Secondary Filters

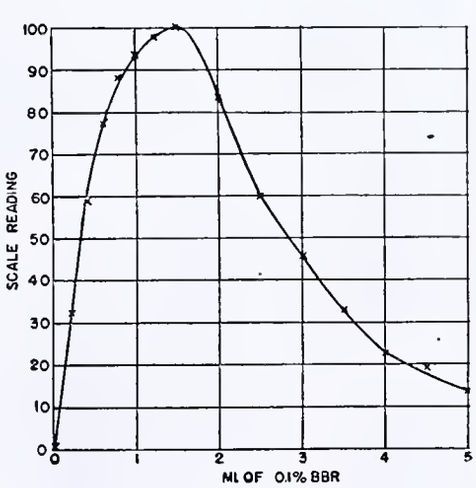

Figure 3. Effect of Blue Black R Concentration on Fluorescence Intensity

Table I. Analysis of Synthetic Standard Steels

Al Present %	Scale Reading	Al Found %
0.000 Standard	0.0	...
0.050 Standard	50.0	...
0.001	1.0	0.001
0.026	24.8	0.025
	26.0	0.026
0.051	51.4	0.051
	49.6	0.050
0.076	78.7	0.079

taining 0.020 mg. of aluminum, 0.50 gram of ammonium acetate, 0.05 ml. of acetic acid, and 1.5 ml. of Blue Black R was allowed to stand several hours, and its fluorescence was measured at room temperature, 25° C. Then it was cooled in ice, and the intensity measured at 10°, 15°, and 20° C. The fluorescence increased slightly at the lower temperatures. The reading was about 2% greater at 10° than at 25° C.

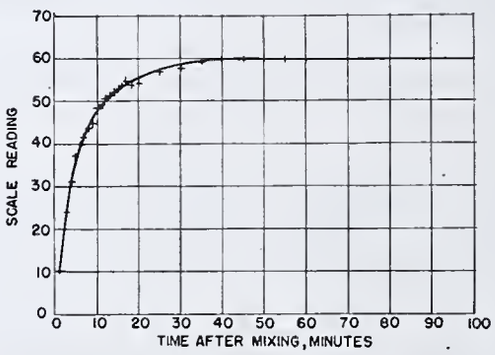

Figure 4. Time Required to Attain Full Intensity of Fluorescence

The experimental conditions thus determined for maximum reproducibility and convenience were: 1.5 ml. of 0.1% Blue Black R in a 50-ml. volume, a pH about 4.8 resulting from 0.50 gram of ammonium acetate plus 0.05 ml. of sulfuric or acetic acid, mixing at room temperature, and letting stand at least one hour before measuring.

Concentration of Aluminum. To determine how nearly the flourescence intensity is a linear function of aluminum concentration, a series of 20 solutions was made up. Each contained 0.50 gram of ammonium acetate, 1.5 ml. of Blue Black R, 0.06 ml. of acetic acid, and aluminum varying from 0 up to 0.100 mg., in a volume of 50 ml., and was let stand for 2 hours before reading. As can be seen from Figure 6, the fluorescence increases in a fairly linear manner until a saturation point is reached at about 0.050 mg. of aluminum, beyond which the intensity remains almost perfectly constant.

The flat portion of this curve is only slightly affected by the aluminum-dye ratio for small increases of dye beyond 1.5 ml. The addition of more dye causes an increase in absorption of both the ultraviolet and the emitted light and gives a reduction in all the readings. The use of 2 ml. of dye extends the useful portion of the curve to 60 micrograms, but greatly reduces the accuracy of the lower concentrations.

The experiment was repeated, except that irradiation by higher energy ultraviolet was used, by substituting Corning No. 9863 for the usual No. 5874 as the primary filter. The former transmits down to 2300 Å., while the latter, which was used for all the other experiments, transmits only down to 3200 Å. The results obtained were very similar to the previous ones, except that the plateau appeared at a slightly higher concentration, 0.060 mg.

A similar set was prepared which covered the range from 0 up to 0.020 mg. Figure 7 shows that the concentration-intensity relation is practically linear up to 0.020 mg. This was repeated several times; greater deviations were noticed in the summer, when room temperature was about 33° C.

To investigate the lower limit of sensitivity, a series covering the range up to 0.001 mg. was prepared. Although a fairly linear graph was obtained, this was not completely reliable because of the rapid decrease in the weak fluorescence during the period of irradiation. However, it was easy even visually to distinguish among solutions containing 0.0004 and 0.0008 mg. of aluminum in the 50-ml. volume. This corresponds to a sensitivity of 1 part in 125,000,000.

Nature of the Fluorescent Substance. To determine if possible the formula of the fluorescent complex, a solution of 5 grams of Blue Black R dyestuff and 25 grams of potassium aluminum sulfate crystals in 300 ml. of water was adjusted to pH about 5.0, by the addition of solid ammonium acetate, and allowed to stand a few hours. It was then extracted with successive 100-ml. portions of *n*-amyl alcohol, and the strongly fluorescent extracts were carefully evaporated to dryness. Analysis of the dried residue indicated that two dye molecules are combined with each aluminum hydroxide molecule. Calculated for $AlOHO_2$-$(C_{20}H_{11}N_2SO_3Na)_2$: C, 56.74%; H, 2.74%; Al, 3.19%. Found: C, 56.64, 58.13%; H, 4.30, 4.58%; Al, 3.19, 3.21%

Analysis of Synthetic Standard Steels. A convenient means for quantitatively determining very small amounts of aluminum in pure solutions had been established by the foregoing work; it still remained to apply the technique to the analysis of actual materials, such as steel. Therefore, varying percentages of aluminum were added to NBS iron 55a, and the resulting synthetic standards were analyzed by Method A, below. Good results were obtained, as shown in Table I.

Analysis of NBS Standard Steels. Several Bureau of Standards steels were analyzed by Method A for acid-soluble aluminum content, which is of greater metallurgical interest than total aluminum. Table II shows the accuracy attained for both high and low percentages. It may be noted that the fluorescence method offers major advantages in the analysis for low percentages, rather than high. Acid-insoluble alumina in steel may also be determined with accuracy (Table III).

Analysis of NBS Standard Bronzes. Several Bureau of Standards copper-base alloys, covering a range of aluminum content, were analyzed by Method B, below. The variability of the results (Table IV) is less than that on the certificates of standardization.

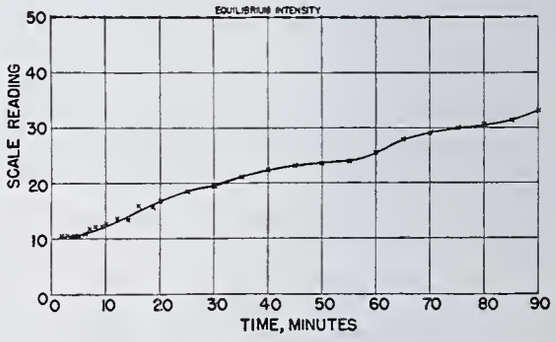

Figure 5. Time Change of Fluorescence after Heating to 70° C.

Analysis of NBS Standard Minerals. Three Bureau of Standards minerals were analyzed for alumina by Method C, below. Again, good accuracy and small dispersion of results were achieved (Table V).

METHODS IN DETAIL

Method A, for Steels. To determine acid-soluble aluminum, dissolve a 1.000-gram sample in 25.0 ml. of 1 to 9 sulfuric acid, and dilute to 500 ml. without filtration. (If the aluminum content is over 0.10%, use a 0.100-gram sample.) Electrolyze a 10-ml. aliquot, containing 0.020 gram of steel, for 1 hour in a small motor-stirred Melaven mercury cathode cell (7) at a current of 0.5 ampere or slightly less. Draw off the solution and rinsings

into a 50-ml. volumetric flask which contains 5.0 ml. of 10% ammonium acetate plus 1.50 ml. of 0.1% Blue Black R, and dilute to the mark. After at least 1 hour, measure and compare the fluorescence intensity of the solutions and of pure aluminum standards prepared similarly, including electrolysis, and containing the same amounts of acid, buffer, and dye as the unknowns. Inasmuch as the calibration curve is nearly linear, only two standards are required, except in most accurate work.

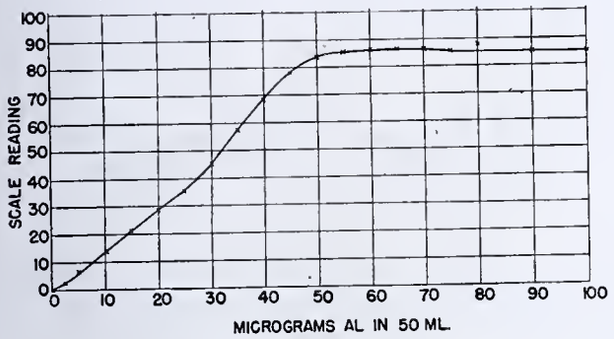

Figure 6. Dependence of Blue Black R Fluorescence on Aluminum Concentration

To determine the acid-insoluble alumina in steel, filter off whatever insoluble residue remains after dissolving a 1.000-gram sample in 1 to 9 sulfuric acid, using a retentive paper, and wash thoroughly with 5 to 95 hydrochloric acid and then water. Ignite in a platinum crucible until all carbon is gone, then add 1 ml. of 1 to 1 sulfuric acid and 5 ml. of hydrofluoric acid. Evaporate down to copious fumes of sulfuric acid, allow to cool, wash down the sides of the crucible with a few milliliters of water, and evaporate and fume strongly again. When cool, transfer to a large platinum dish with 50 ml. of water, and heat until the salts dissolve completely. Add 10% sodium hydroxide solution until alkaline to methyl red, then 1 to 9 sulfuric acid dropwise until barely acid, then an excess of 25.0 ml. of 1 to 9 sulfuric acid. Transfer to a 500-ml. volumetric flask, dilute to the mark, shake well, and pipet out a 10-ml. aliquot for mercury cathode electrolysis. Carry out the remainder of the analysis in the usual manner.

Method B, for Bronzes. Weigh out a 1.000-gram sample, for materials containing 0.10% aluminum or less; for higher percentages, up to about 1%, use a 0.100-gram sample. Dissolve in 10 ml. of 1 to 1 nitric acid in a 250-ml. beaker, add 20 ml. of 1 to 1 sulfuric acid, and evaporate down to copious fumes of sulfuric acid. Allow to cool, wash down the sides with about 15 ml. of water, and again evaporate to strong fumes. (Each evaporation may be accomplished in a few minutes by heating the beaker over a free flame, with vigorous swirling of the contents.) Cool, dilute with 100 ml. of water, make just neutral to methyl red with 10% sodium hydroxide, and add 25.0 ml. of 1 to 9 sulfuric acid. Dilute to 500 ml., shake well, and pipet a 10-ml. aliquot into the mercury cathode cell. After electrolyzing for an hour, draw off into a 50-ml. volumetric flask containing 5.0 ml. of 10% ammonium acetate and 1.50 ml. of 0.1% Blue Black R, and dilute to the mark. Let stand at least 1 hour, and then measure the fluorescence and compare with that of standards prepared similarly and simultaneously.

Table II. Analysis of Bureau of Standards Steels for Acid-Soluble Aluminum

Steel	Certified Value %	Found %
55a	0.001	0.001 0.001 0.001 0.001
4c	0.022	0.021 0.021 0.024 0.022 0.022 0.021 0.021 0.022
106a	1.07	1.05 1.05 1.12 1.13
106	1.06 (total)	1.09 1.09 1.05 1.05

Table III. Analysis of Bureau of Standards Steel for Acid-Insoluble Alumina

Steel	Certified Value %	Found %
106a	0.011	0.011 0.011 0.011 0.012

Table IV. Analysis of Standard Bronzes for Aluminum

Alloy	Certified Value %	Found %
62	1.13	1.09 1.14 1.11
62a	0.92	0.93 0.93 0.95 0.96
62b	0.97	0.98 0.95 0.95 0.97
63	0.05	0.044 0.051 0.052 0.055
124a	0.006	0.006 0.008

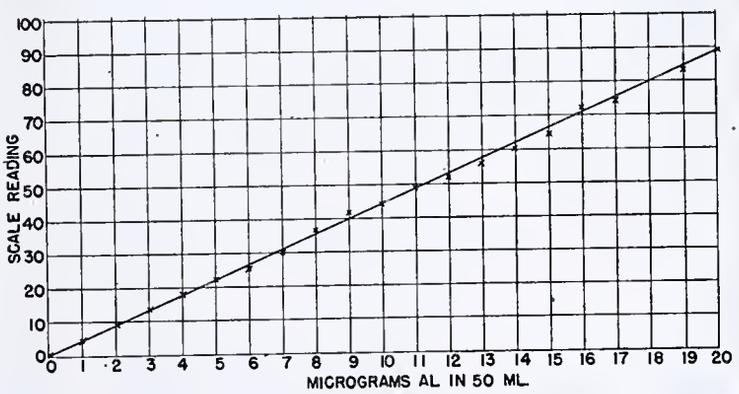

Figure 7. Dependence of Blue Black R Fluorescence on Concentration

Method C, for Minerals. For materials consisting largely of silica, such as glass sand or silica brick, the following direct method is suitable. Weigh 0.100 or 0.500 gram of sample (depending on aluminum content) into a 20-ml. platinum crucible, add 2 ml. of 1 to 1 sulfuric acid and 10 ml. of hydrofluoric acid, and evaporate down to copious fumes. Cool, wash down the sides with 10 ml. of water, and again evaporate to heavy fumes. Add to 100 ml. of water in a platinum dish, heat until all salts dissolve, and make just neutral to methyl red with 10% sodium hydroxide. Then add 25.0 ml. of 1 to 9 sulfuric acid, dilute to 500 ml., and electrolyze a 10-ml. aliquot in the Mclaven cell for an

Table V. Analysis of Standard Minerals for Alumina

Sample	Certified Value %	Found %
81	0.265	0.268 0.279 0.287
88	0.067	0.063 0.067 0.063 0.062
102	1.96	1.98 2.01 2.03

hour. Complete the determination as described in Methods A and B, and calculate the results as aluminum oxide. The blank on the complete procedure is very small, about 0.0001 mg. of aluminum oxide on the 10-ml. aliquot.

For other types of minerals, it is more convenient to proceed with the ordinary scheme of analysis, and then apply the fluorescence method to the determination of alumina in the R_2O_3 ammonia precipitate. This may be illustrated in the case of NBS dolomite No. 88.

Ignite a 1.000-gram sample at 1100° for 30 minutes, then boil with 40 ml. of 1 to 1 hydrochloric acid. Filter off the small insoluble residue, wash, ignite in a platinum crucible, treat with 5 drops of 1 to 4 sulfuric acid and 5 ml. of hydrofluoric acid, and evaporate to dryness. Then fuse with 0.5 gram of potassium pyrosulfate, dissolve the cooled melt in water, and add this solution to the original filtrate and washings. Add about 10 grams of ammonium chloride and precipitate the R_2O_3 as usual with ammonia, using methyl red indicator. Filter off the hydroxides, wash several times, transfer to a beaker by a jet of water, and dissolve by the addition of 25.0 ml. of 1 to 9 sulfuric acid. Then dilute the solution to 500 ml., pipet out a 10-ml. aliquot for electrolysis, and complete the determination in the usual manner.

DISCUSSION

The mercury cathode separation was chosen as most suitable here for removing iron, copper, etc.; even a little ferric ion destroys fluorescence. Attempts were made to extract the fluorescent complex from the iron solution with amyl alcohol, and also to use sodium hydroxide or cupferron for eliminating the iron, but none of these gave as good results as the mercury cathode.

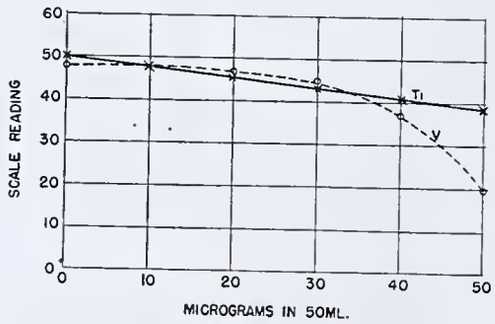

Figure 8. Interference by Titanium and Vanadium in Solutions Containing 0.010 Mg. of Aluminum

Such interferences as iron, copper, chromium, nickel, and cobalt are removed quantitatively by mercury cathode electrolysis. It was of interest to investigate possible interference by titanium, vanadium, and zirconium, which are not so removed and which are frequently found in steel. Varying quantities of these ions were added to fluorescing solutions which contained 0.010 mg. of aluminum. Titanium was found to interfere somewhat, and pentavalent vanadium more seriously (Figure 8), but zirconium,

Table VI. Determination of Aluminum in Titanium and Vanadium Steels

Steel	Gravimetric Analysis	Aluminum Found %
K	0.066% Al, 0.085% Ti	0.064
		0.066
		0.064
		0.063
L	0.051% Al, 0.11% V	0.053
		0.053
		0.053
		0.054

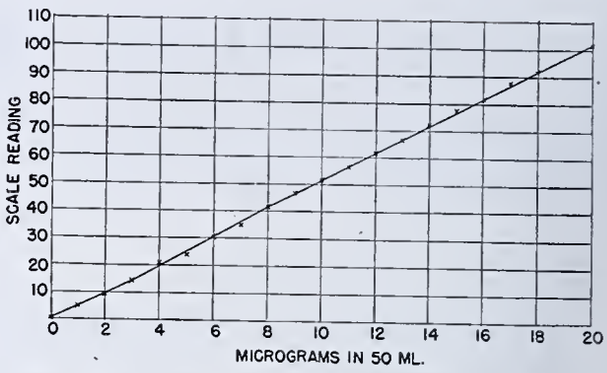

Figure 9. Dependence of Pontachrome Violet SW Fluorescence on Aluminum Concentration

not at all. Nevertheless, good results were obtained in the fluorometric determination of aluminum in titanium and vanadium steels (Table VI), perhaps because the carbides of the latter are insoluble in dilute sulfuric acid.

Pontachrome Violet SW has also been suggested (*9*) as a fluorescence reagent for aluminum; it gives an orange fluorescence at 5850 to 6250 Å, compared with the red fluorescence of Blue Black R at 6365 to 7000 Å. In the Violet SW dye molecule, a benzene ring replaces one of the naphthalene rings in Blue Black R.

Detailed investigations of the suitability of Violet SW as a quantitative reagent showed that wider variations in dye concentration could be tolerated, that the dependence of intensity on aluminum concentration was almot perfectly linear (Figure 9), and that accurate results could be obtained for amounts of aluminum as small as a few tenths of a microgram. However, the Violet SW fluorescence required more careful control of pH, and was destroyed by even smaller traces of iron. Therefore, the Blue Black R reagent was chosen for the bulk of this work, although analysis of NBS and synthetic standard steels were completed with good accuraey, using Violet SW.

ACKNOWLEDGMENT

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Quantitative Determination of Some Inhibitors in Polymers by Ultraviolet Light Absorption

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A rapid and accurate method of analysis for inhibitors in polymeric materials employing ultraviolet light absorption has been developed which is especially desirable for inhibitors present in small concentrations. Correction for linear "background" absorption is made without need for direct measurement.

ORGANIC polymeric substances are commonly subject to attack by atmospheric oxygen, which will cause changes in the physical properties of the polymers. Inhibitors are usually incorporated to prevent or retard polymer degradation caused by atmospheric and imposed oxidizing conditions. The use of stabilizers is, therefore, very widespread and is especially important in synthetic rubbers.

Inhibitor concentration is an important factor in estimating the potential stability of a polymer. The concentration requirements for stabilizing polymers of different types will vary with the type of polymer. The use of small concentrations of inhibitor has created an urgent need for a more precise method of inhibitor analysis. Production control requires an easy and rapid method of analysis as well as accurate results, even where higher concentrations of inhibitor are used. *N*-Phenyl-2-naphthylamine (commonly called phenyl-β-naphthylamine) has been used very widely in the stabilization of synthetic elastomers and, therefore, an effort was made to develop the best analytical method for this specific inhibitor. A method of analysis of primary aromatic amines involving titration with standardized nitrous acid (*5*) has been applied to its determination; however, when this method was used to determine the inhibitor present in a polymer in small concentrations, it was found unreliable in the absence of very careful control and expert manipulation. Craig (*2*) estimated the concentration of *N*-phenyl-2-naphthylamine in rubber by isolation of the hydrochloride. An adaptation of this method to Butyl rubber by a turbidimetric measurement of the amine hydrochloride has been used for production analysis but was unsatisfactory unless very careful control was maintained. An oxidative method using ceric sulfate has also been used with somewhat more reliable results. Investigation of rapid physical methods of analysis revealed that *N*-phenyl-2-naphthylamine has a very strong absorption in the near-ultraviolet region (*4*, *6*, *7*) which is very desirable for spectral analysis. Qualitative spectrographic determination of various rubber ingredients, including *N*-phenyl-2-naphthylamine, has been described (*3*). The use of spectrophotometry now appears to be the best method of analysis for *N*-phenyl-2-naphthylamine in polymers.

Since polymers and various blending ingredients are not entirely transparent to ultraviolet light, it is necessary to subtract the "background" absorption (absorption due to materials other than *N*-phenyl-2-naphthylamine) to obtain a true estimation of inhibitor concentration in the polymer. A mathematical adaptation of the method which Wright (*8*) used in infrared spectroscopy in correcting for the background absorption, without actual measurement of the background absorption, was applied to this determination with considerable success. The method can be satisfactorily used with any polymer which can be dissolved in a solvent, or from which the inhibitor can be extracted by a solvent, where the background absorption is linear through the spectral points or wave lengths at which the inhibitor exhibits a maximum and two adjacent minimum absorptions. The correction for linear background absorption by optical density measurements at three wave lengths is outlined below. Although certain forms of this mathematical approach are in use, to the authors' knowledge it has not been previously reported. (Derivation and first application of this equation to polymer analysis was carried out in this laboratory by T. S. Chambers.)

Two assumptions are made: (1) Beer's law is obeyed and (2) the background absorption is linear with wave length over the portion of the spectrum involved. The former assumption has been found valid for the concentrations employed. Knowledge of the system used in the analysis will determine whether the latter is true or is a close approximation which would introduce but little error. Figure 1 is a typical illustration of the absorption of a solution of polymer containing an inhibitor.

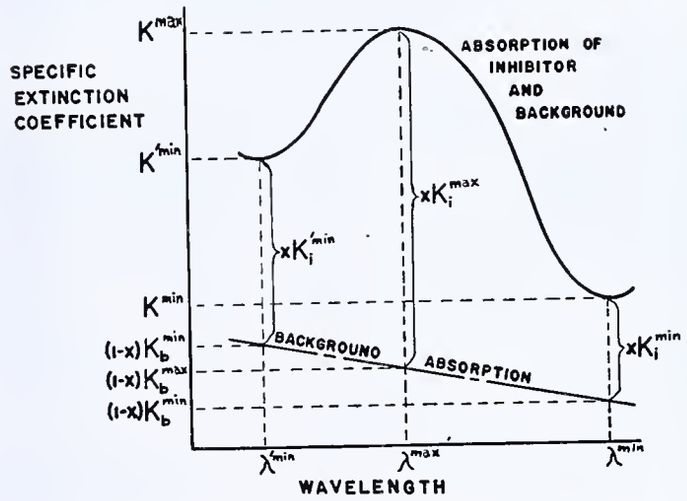

Figure 1. Typical Absorption of Solution of Polymer Containing an Inhibitor

K'^{min}, K^{max}, and K^{min} are observed specific extinction coefficients of the polymer sample being analyzed for inhibitor content. $K_b'^{min}$, K_b^{max}, and K_b^{min} are the specific extinction coefficients of the background and $K_i'^{min}$, K_i^{max}, and K_i^{min} are the specific extinction coefficients for the inhibitor. Let x = fraction of inhibitor in polymer; and $1 - x$ = fraction of polymer or background.

Then

$$K'^{min} = xK_i'^{min} + (1 - x)\,K_b'^{min} \quad (1)$$

$$K^{max} = xK_i^{max} + (1 - x)\,K_b^{max} \quad (2)$$

$$K^{min} = xK_i^{min} + (1 - x)\,K_b^{min} \quad (3)$$

The K's and K_i's are experimentally determined, leaving four unknowns. The value of x can be determined without knowing K_b's if one of the K_b's is known in terms of the others. From Figure 1:

$$(1 - x)K_b^{max} \doteq (1 - x)K_b^{min} + \frac{\lambda^{min} - \lambda^{max}}{\lambda^{min} - \lambda'^{min}}[(1 - x)K_b'^{min} - (1 - x)K_b^{min}] \quad (4)$$

or

$$K_b^{max} = (1 - n)K_b^{min} + nK_b'^{min} \quad (5)$$

where

$$n = \frac{\lambda^{min} - \lambda^{max}}{\lambda^{min} - \lambda'^{min}} \quad (6)$$

Substitute values of $K_b'^{\min}$ and $K_b^{\min}$ from Equations 1 and 3 in Equation 5:

$$K_b^{\max} = \frac{(1-n)(K^{\min} - xK_i^{\min}) + n(K'^{\min} - xK_i'^{\min})}{1-x} \quad (7)$$

Substitute value of $K_b^{\max}$ from Equation 7 in Equation 2:

$$x = \frac{K^{\max} - (1-n)K^{\min} - nK'^{\min}}{K_i^{\max} - (1-n)K_i^{\min} - nK_i'^{\min}} \quad (8)$$

Since $K = \frac{D}{LC}$

where D = optical density = $\log \frac{I \text{ solvent}}{I \text{ solution}}$

L = cell length = 1 cm.

C = concentration of polymer in solution in grams per liter

I solvent = intensity of light through pure solvent

I solution = intensity of light through polymer solution

then

% inhibitor in polymer =

$$\frac{100[D^{\max} - (1-n)D^{\min} - nD'^{\min}]}{C[K_i^{\max} - (1-n)K^{\min} - nK_i'^{\min}]} \quad (9)$$

EXPERIMENTAL

The choice of solvent should be such that it will dissolve both polymer and inhibitor and it must be easily obtained in a pure state and preferably show low and linear absorption in the spectral region employed. Ethylene dichloride and chloroform were satisfactory solvents for GR-S and Perbunan. These solvents as well as carbon tetrachloride and iso-octane were suitable solvents for Butyl rubber.

A Beckman quartz prism spectrophotometer (*1*) with a hydrogen discharge tube serving as the light source was used for all optical density measurements. Quartz cells with a light path of 1 cm. held the liquid samples.

Extinction coefficients of the inhibitors, in the solvents to be used for the analyses, were determined by optical measurements at the wave lengths of the maximum and two adjacent minimum absorptions best suited for analytical purposes. Several concentrations were employed and an average extinction coefficient was calculated for the inhibitor at each of the three wave lengths. It is important that measurements of extinction coefficients of inhibitors be made for each instrument, since the exact dispersion, resolving power, actual slit width, photocell spectral sensitivity, and amount of reflected and scattered light of the instrument are factors which affect the optical density measurements.

Since only small samples of polymer were employed, care was exercised to get truly representative results. This was accomplished by milling and refining a sample of rubber from which portions were taken for analyses. Milling was also beneficial in destroying gel in polymers which would introduce error from light reflection. Excessive milling and heating in contact with air were avoided.

An accurately weighed portion of polymer was placed in a glass-stoppered Erlenmeyer flask and a known volume of solvent added from a pipet. Dissolution occurred on standing 1 or 2 days with occasional shaking, or it was accelerated by stirring or heating. A clear sample was removed from any settled insoluble material (centrifuging may be used) and transferred to the quartz cell for optical density measurements. A matched quartz cell containing a solvent control was used as the reference zero optical density. The concentration of inhibitor in the polymer was calculated by means of Equation 9. Using this procedure, only a few minutes are required to determine the inhibitor content of a sample of dissolved polymer.

DISCUSSION

The specific extinction coefficients of *N*-phenyl-2-naphthylamine showed slight variation in numerical value and spectral position with the solvent employed. The evaluation of the coefficients used in calculations in this paper are given in Table I

Table I. Specific Extinction Coefficients of *N*-Phenyl-2-naphthylamine and Uninhibited Polymers

Wave Length, mμ	Specific Extinction Coefficients, K (l. g.$^{-1}$ cm.$^{-1}$)				
	PBN in iso-octane	Butyl rubber in iso-octane	PBN in $C_2H_4Cl_2$	GR-S in $C_2H_4Cl_2$	Perbunan in $C_2H_4Cl_2$
282	49.7	0.01–0.06	54.0	...	...
284	52.0	.....	51.5	0.05	0.03
308	85.6	01–0.04	85.0	...	...
309	83.5	...	87.0	0.04	0.02
326	13.2	0.01	17.0	...	...
332	15.0	.....	15.0	0.01	0.01

and their use permits simplification of Equation 9 to the following expressions:

% PBN in Butyl rubber =

$$(D^{308} - 0.6D^{326} - 0.4D^{282})\frac{1.73}{C} \quad (10)$$

% PBN in Perbunan and GR-S =

$$(D^{309} - 0.52D^{332} - 0.48D^{284})\frac{1.83}{C} \quad (11)$$

where the solvent for Butyl rubber is iso-octane and that for Perbunan and GR-S is ethylene dichloride. Solutions containing about 0.01 gram of *N*-phenyl-2-naphthylamine per liter (or about 0.3 gram of Butyl rubber or 0.05 gram of Perbunan or GR-S per 100 ml.) were most satisfactory for optical density measurements with the Beckman instrument. These solutions have optical densities in the range of 0.1 to 1.0 for the wave lengths prescribed.

The advantage of the ultraviolet absorption method for the determination of *N*-phenyl-2-naphthylamine in Butyl rubber as compared with the turbidimetric determination by precipitated hydrochloride is evident from Table II. An oxidation method involving ceric sulfate oxidation of the inhibitor gave results in good agreement with those obtained by the ultraviolet method.

Table II. Comparison of Methods of Analysis of Butyl Rubber for *N*-Phenyl-2-naphthylamine

Polymer Composition	% *N*-Phenyl-2-naphthylamine in Polymer			
	Chemical Methods		Ultraviolet Method	
	Hydrochloride turbidimetric	Ceric sulfate	Corrected for background	Uncorrected for background
Pure Butyl rubber	0.00	...	0.00	0.01
Pure Butyl rubber + 0.25% PBN[a]	0.22	...	0.24	0.26
Pure Butyl rubber + 2.5% zinc stearate[a]	0.00	...	0.00	0.03
Pure Butyl rubber + 2.5% zinc stearate + 0.25% PBN[a]	0.40	...	0.23	0.26
Butyl rubber (light colored)	0.63	0.35	0.35	0.41
Butyl rubber (dark colored)	0.48	0.00	0.00	0.09
Butyl rubber (dark colored) + 0.25% PBN[a]	0.42	0.25	0.21	0.33

[a] Added on mill.

The calculation of inhibitor content by using only the wave length of maximum absorption gives values listed in Table II as uncorrected for background. This calculation represents the maximum amount of inhibitor that can be in the polymer and is made by Equation 12:

Maximum possible % PBN in polymer =

$$\frac{100K^{308}}{K_i^{308}} = 1.17\frac{D^{308}}{C} \quad (12)$$

The uncorrected value does not always agree with that corrected for background absorption but tends to be higher where the background absorption is greater.

The dark-colored Butyl rubber sample in Table II showed no inhibitor present by corrected ultraviolet analysis, while the un-

Table III. Comparison of Different Methods of Analysis of Buna-Type Polymers for *N*-Phenyl-2-naphthylamine

Polymer Composition[a]	% *N*-Phenyl-2-naphthylamine in Polymer: Ultraviolet Method, Corrected for background	Ultraviolet Method, Uncorrected for background	Chemical method, nitrous acid titration of HOAc extract
Unstabilized Perbunan	0.00	0.02	0.00
Perbunan + 1.9% PBN added on mill (A)	1.91	1.99	...
Perbunan (A) milled 10 minutes at 20° C.	1.88	1.96	...
Perbunan (A) milled 10 minutes at 100° C.	1.80	1.96	...
Perbunan (A) aged 4 days, air oven, 85° C.	1.79	1.87	...
Perbunan + 2.0% PBN added on mill (B)	1.98	2.05	1.91
Perbunan commercial product (C)	1.64	1.69	1.65
Perbunan (C) aged 46 days, air oven, 60° C.	1.32	1.42	...
Perbunan, dark colored stock	1.85	2.26	...
Unstabilized GR-S	0.00	0.05	...
GR-S + 0.5% PBN added on mill (A)	0.51	0.54	0.58
GR-S + 1.96% PBN added on mill (B)	1.96	1.99	1.98
GR-S (B) aged 46 days, air oven, 60° C.	0.74	1.24	...

[a] Parenthetical letter refers to specific polymer-inhibitor compositions.

Even fresh polymers may, however, become contaminated with impurities by milling or improper washing, so that the magnitude of the background absorption is increased, thereby making a background correction necessary if accurate and reproducible results are required. In Table III data are presented illustrating the application of ultraviolet absorption and Equations 11 and 13 to the determination of *N*-phenyl-2-naphthylamine in Buna-type polymers. In certain cases the antioxidant was extracted from the polymers with glacial acetic acid and the extracts titrated with nitrous acid to starch-iodide end points. In these cases special precautions were taken to obtain complete extraction and avoid losses which generally accompany this type of analysis.

Data are also presented in Table III to show the effect of natural and imposed oxidizing conditions on the antioxidant contents of polymers. Ultraviolet analyses of such polymers show not only decreased amounts of antioxidant present in the aged polymers, but also a variation in the two methods of calculation (Equations 11 and 13) which can be accounted for only by assuming that oxidation products of the inhibitor and polymer are contributing to the background absorption.

Inspection of the absorption spectra of highly oxidized Perbunan and GR-S polymers reveal that the characteristic peak absorption of the *N*-phenyl-2-naphthylamine at 309 mμ has been at least partially destroyed, and the absorption has become more linear with wave length than is the case for the absorption of the pure inhibitor. Under such circumstances, application of a background correction results in much more accurate evaluations of the inhibitor contents of the polymers than either measurement of the optical density of the polymer solution at only one wave length, or application of analytical procedures which depend on the reaction of a functional group of the inhibitor. The nitrous acid titration procedure, for example, fails to distinguish oxidation products of *N*-phenyl-2-naphthylamine from the pure inhibitor if amine groups are present in the oxidation products and if these groups will form nitroso derivatives. The work of Rehner, Banes, and Robison (7) has demonstrated that amine compounds may occur in oxidation products of *N*-phenyl-2-naphthylamine.

Table IV. Effect of Aging of Buna-Type Polymers on Ultraviolet Absorption and Inhibitor Analyses

Polymer	Aging Conditions	Optical Density: 284 mμ	309 mμ	332 mμ	Concn., G./L.	% *N*-Pbenyl-2-naphthylamine in Polymer: Ultraviolet Method, Corrected for background	Ultraviolet Method, Uncorrected for background	Chemical method, nitrous acid titration of HOAc extract
Perbunan	Original	0.447	0.732	0.136	0.500	1.64	1.69	...
	50 days, air oven, 82° C.	0.206	0.313	0.076	0.500	0.27	1.05	...
GR-S	Original	0.485	0.805	0.148	0.500	1.81	1.85	1.82
	50 days, air oven, 82° C.	0.360	0.333	0.117	0.500	0.36	0.77	0.43
GR-S	Original solution	0.158	0.878	0.545	0.500	1.96	2.02	...
	Same solution aged 10 days in diffused sunlight at room temperature	0.202	0.716	0.642	0.500	1.11	1.65	...

corrected analysis indicated that inhibitor was still present. Storage stability of this polymer was very poor, indicating that inhibitor was absent.

From a large number of inhibitor determinations on Butyl rubber containing about 0.25% *N*-phenyl-2-naphthylamine, the following probable errors were obtained when corrected for background absorption:

Mean deviation from theoretical	±0.01% PBN
Maximum deviation from theoretical	±0.04% PBN
Mean reproducibility	±0.01% PBN
Maximum deviation from mean	±0.04% PBN

The ultraviolet absorption of GR-S and Perbunan polymers which contain small amounts of such impurities as hydroquinone, soap, fatty acid, persulfate, and mercaptan is very low as compared to that of *N*-phenyl-2-naphthylamine. Since larger amounts of antioxidant are used in the Buna-type polymers (about 2.0%) than in Butyl rubber (about 0.25%), the background absorption of unaged Buna-type polymers is of less significance in determining the inhibitor contents of the polymers by ultraviolet absorption. It is then usually necessary to measure the absorption of a polymer solution only at the wave length of maximum absorption (309 mμ) and calculate the inhibitor concentration by Equation 13.

Maximum possible % PBN in Perbunan or GR-S =

$$\frac{100K^{309}}{K_i^{309}} = 1.15\,\frac{D^{309}}{C} \qquad (13)$$

The changes in ultraviolet absorption and inhibitor content of Perbunan and GR-S polymers due to oxidation promoted by heat and light are further illustrated in Table IV. The more

Table V. Wave Lengths Applicable for Ultraviolet Determination of Inhibitors

Inhibitor	Solvent	Wave Lengths in mμ for Analyses: λ'^{min}	λ^{max}	λ^{min}
Aminox[a]	Ethylene dichloride	246	291	355
Agerite Stalite[a]	Ethylene dichloride	251	286	353
Inhibitor 8567[a]	Iso-octane	246	277	300
N-Phenyl-2-naphthylamine	Iso-octane	282	308	326
N-Phenyl-2-naphthylamine	Ethylene dichloride	284	309	332
N-Phenyl-2-naphtbylamine	Chloroform	284	309	332

[a] Aminox, Amine, ketone condensation product. Agerite Stalite, alkylated aromatic amine. Inhibitor 8567, phenolic type.

probably correct analyses are those obtained where background corrections have been made.

This method of analysis has been extended to other inhibitors. It is most reliable where large values for maximum extinction coefficients are encountered. The wave lengths which were used for analyses for certain of these inhibitors in polymers are shown in Table V.

CONCLUSION

A spectrophotometric method of analysis for inhibitors in polymers has been developed. This method corrects for background absorption and has been found to be rapid and more reliable than chemical methods of analyses which were investigated.

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Analysis of Binary Mixtures of Normal Aliphatic Dibasic Acids and Esters[1]

Use of Composition–Melting Point Relations of the Acids

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An empirical method is presented for determining the melting ranges of fused and quenched samples of dibasic acid mixtures. Application to binary mixtures of alternate and adjacent acids containing six to twelve carbon atoms reveals that compositions may be determined within 1 to 5%, depending on the composition of the sample. Approximate data on eutectic temperatures of the systems are included.

MIXTURES of similar dibasic acids often occur in oxidation products from unsaturated fatty acids, and existing methods for their separation and analysis are somewhat unsatisfactory. Crystallizations yield, with difficulty, only small proportions of pure components. The precision of saponification or neutralization equivalent determinations is limited by the 7-unit difference in equivalent weights of adjacent dibasic homologs. Vacuum fractionation of esters gives perhaps the most effective separation, but boiling points of adjacent esters are rather close, and intermediate fractions inevitably remain. Additional composition-property data are desirable for corroborative purposes.

The results of Gantter and Hell (*1*) for melting points of binary mixtures of suberic and azelaic acids indicated that this property might prove useful. The data, though irregular, showed that a marked melting point depression occurred in the system, and hence a considerable range of values was available. The lack of further published information for similar systems appears traceable to the fact that powdered binary mixtures of dibasic acids frequently melt over wide temperature ranges without distinct relation to composition.

An empirical method has now been developed for taking melting points which provides results closely related to composition and affords its determination within a few per cent. This method has been applied to the systems comprising adjacent and alternate pairs of acids containing six to twelve carbon atoms.

PREPARATION OF SAMPLES

Acids used are presented in Table I. One-gram samples of mixtures were prepared by weighing the component acids to 0.2 mg., bringing the mixtures to complete fusion, stirring them during cooling, and powdering the solidified material in a mortar. Portions were introduced into capillary tubes 1.0 to 1.5 mm. in diameter and liquefied by holding the tubes in a stirred and rapidly heated oil bath. Air bubbles, which frequently developed, were dislodged by momentarily removing the tubes and shaking them sharply as with a clinical thermometer. The melted samples were quenched by quickly transferring the capillary tubes to a stream of tap water. Quenched samples were 3 to 5 mm. in height. Approximate melting ranges were observed during the fusion process and allow rapid temperature adjustment in taking the final measurements.

Table I. Dibasic Acids Used for Binary Mixtures

No. of Carbon Atoms	Acid	Capillary M. P., ° C.	Source
6	Adipic	152.0	Eastman product as received
7	Pimelic	103.8–104.3	
8	Suberic	141.4–141.9	Prepared from castor oil acids by oxidation with nitric acid
9	Azelaic	106.6–107.0	
10	Sebacic	132.8–133.1	Eastman product, recrystallized
11	1,11-Undecanedioic	110.3–110.8	From 12-hydroxystearic acid by method of Hall and Reid (*2*)
12	1,12-Dodecanedioic	128.7–129.0	

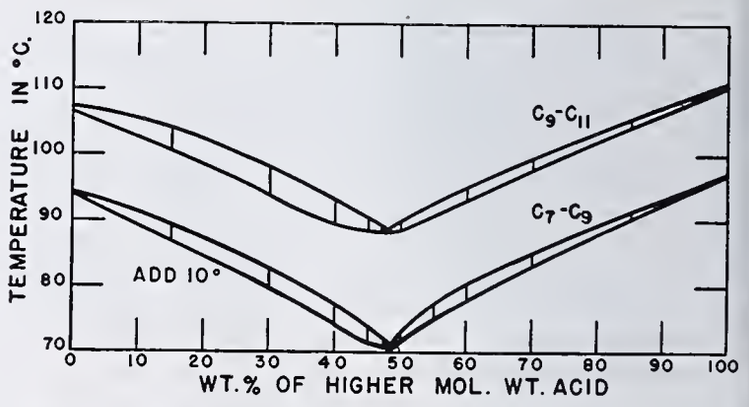

Figure 1. Composition–Melting Point Curves for Binary Mixtures of Odd-Carbon Dibasic Acids

Temperature scale to be shifted as indicated for individual curves

[1] Second article on this subject appears on page 541.
[2] Present address, F. E. Booth Co., Emeryville, Calif.

Table II. Melting Ranges of Binary Mixtures of Alternate Dibasic Acids

Weight % of Higher Molecular Weight Acid	Odd-Odd		Even-Even		
	$C_7 + C_9$	$C_9 + C_{11}$	$C_6 + C_8$	$C_8 + C_{10}$	$C_{10} + C_{12}$
0	103.8–104.3	106.6–107.0	152.0	141.4–141.9	132.8–133.1
5	101.4–103.1	104.6–106.7	149.4–150.8	139.3–140.5	131.0–132.7
10	99.2–101.4	102.4–105.7	147.1–149.5	137.0–138.8	129.0–131.9
15	97.1– 99.6	100.4–104.1	144.3–147.9	134.2–136.9	127.0–130.9
20	94.5– 97.5	98.4–102.4	141.9–146.2	132.0–134.9	124.9–129.8
25	92.2– 94.9	96.3–100.4	139.5–144.3	129.3–132.7	122.8–128.4
30	89.5– 92.7	94.1– 98.2	136.8–142.1	126.5–130.4	120.6–126.6
35	87.0– 90.0	91.7– 95.9	134.0–139.8	123.7–127.7	117.8–123.6
40	84.3– 87.4	89.4– 93.4	131.1–137.4	120.4–124.6	115.0–120.1
45	81.3– 84.1	88.4– 90.6	127.5–133.8	117.5–120.6	113.1–116.0
50	81.3– 82.5	88.5– 89.7	122.8–129.2	114.5–116.1	112.0–112.7
55	84.9– 87.2	90.7– 92.7	120.0–124.1	112.8–113.7	111.6–112.5
60	87.8– 90.1	93.1– 95.1	119.3–119.7	113.0–114.0	112.3–113.7
65	90.4– 92.6	95.4– 97.4	119.8–120.1	115.3–117.2	114.0–116.8
70	93.0– 94.8	97.7– 99.3	122.2–124.1	118.6–121.6	116.9–120.3
75	95.4– 97.0	99.8–101.4	124.9–128.5	121.2–124.8	119.2–122.9
80	97.9– 99.2	102.0–103.2	127.7–131.9	123.8–127.4	121.2–124.7
85	100.2–101.4	104.1–105.2	130.5–134.8	126.0–129.5	122.0–125.9
90	102.5–103.4	106.1–107.0	133.4–137.8	128.5–131.2	124.9–127.2
95	104.6–105.3	108.2–108.9	137.3–140.2	130.6–132.3	126.8–128.2
100	106.6–107.0	110.3–110.8	141.4–141.9	132.8–133.1	128.7–129.0

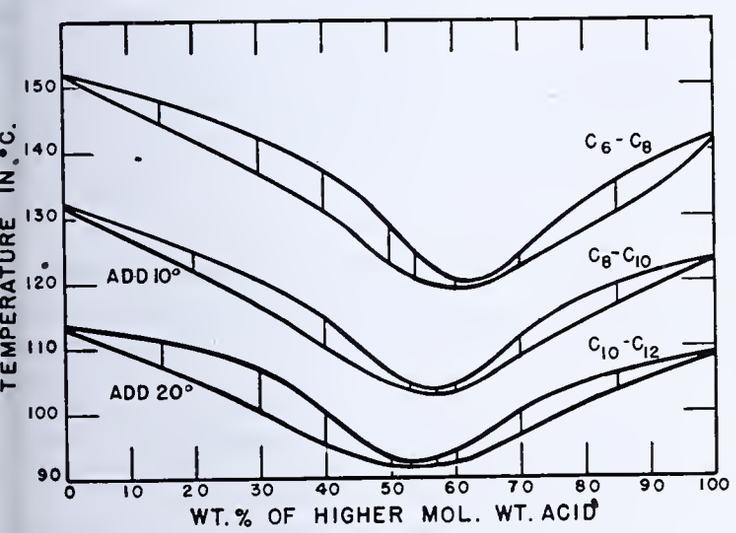

Figure 2. Composition–Melting Point Curves for Binary Mixtures of Even-Carbon Dibasic Acids

Temperature scale to be shifted as indicated for individual curves

DETERMINATION OF MELTING RANGE

Melting ranges were determined in an electrically heated (7), mechanically stirred oil bath. The rate of heating was closely controlled by a variable-voltage transformer. Temperatures were read on a 76-mm. immersion thermometer calibrated in degrees and compared with thermometers having calibrations checked by the National Bureau of Standards. Samples were placed next to the bulb of the thermometer and observed through a magnifier while under transverse illumination. Bath temperatures were brought rapidly (3° to 5° per minute) to a few degrees below the expected melting range, and thereafter increased at not over 0.2° C. per minute. The melting range was recorded from the first appearance of clear liquid to the disappearance of the last solid particle. Clear liquid first appeared as a partial meniscus in most cases, though mixtures which were predominantly one-component sometimes began to melt at other peripheral locations. The lower curves shown in the graphs for the individual systems represent empirically reproducible temperatures for the first appearance of clear liquid, and should not be confused with the solidus curves of binary systems having continuous series of solid solutions.

EUTECTIC PHENOMENA

A marked change occurred in the gross appearance of the quenched samples at temperatures below the melting points for all mixtures in a wide central portion of the composition range. The hard waxy surface assumed a soft matte appearance. The temperature of this phenomenon—as observed during the initial rapid heating—was constant within 2° or 3° for all compositions of a binary system that were not grossly erystalline after quenching. The temperatures varied from system to system as shown in Table IV. Microscopical observations showed that the phenomenon actually consisted in the melting of an eutectic mixture.

The occurrence of a constant eutectic temperature over a portion of the composition range apparently places the binary systems in the class characterized by formation of solid solutions of limited solubility (Roozeboom's Type V).

The differences between these eutectic temperatures and those obtained from "thaw-point" (*5*, *6*) measurements (in which the first apparent changes are observed in powdered samples) probably result from the intentional disturbance of equilibria by quenching. The difference between the eutectic temperatures and the melting ranges of the eutectic compositions obtained from the graphs (Table IV) may be partially due to measurement of different stages of melting, but it is more likely that polymorphism of the acids is involved. For example, in the suberic-azelaic system it was found that the melting range at the eutectic composition was 96.7–97.7° C. by the graphical procedure, but the eutectic temperature over a range of compositions was 91.5–92.5° C. by thaw-point measurement or 94–95° C. by heating fused samples slowly. Kofler (*4*) has shown that suberic acid can exist in three forms, and he reported that the transition from Form I to Form III is enantiotropic, occurs at about 90° C., and is very susceptible to superheating or supercooling. Azelaic acid has also been shown to be at least dimorphic. Polymorphism would likely be encountered in binary mixtures of these acids, especially in connection with intentional departures from equilibrium conditions. These phenomena have not been studied further.

COMPOSITION–MELTING POINT DATA

Systems investigated comprise four classes: with (*a*) alternate, even-carbon acids; (*b*) alternate, odd-carbon acids; (*c*) adjacent acids, with the odd-carbon member of higher molecular weight;

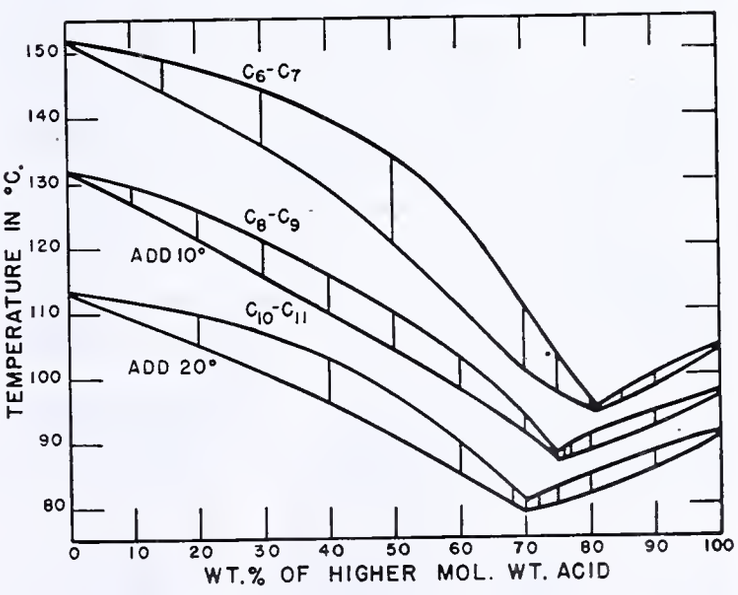

Figure 3. Composition–Melting Point Curves for Binary Mixtures of Adjacent Dibasic Acids with Higher Molecular Weight Odd-Carbon Acid

Temperature scale to be shifted as indicated for individual curves

Table III. Melting Ranges of Binary Mixtures of Adjacent Dibasic Acids

Weight % of Higher Molecular Weight Acid	Odd-Carbon Acid of Higher Molecular Weight			Even-Carbon Acid of Higher Molecular Weight		
	$C_5 + C_7$	$C_8 + C_9$	$C_{10} + C_{11}$	$C_7 + C_8$	$C_9 + C_{10}$	$C_{11} + C_{12}$
0	152.0	141.4–141.9	132.8–133.1	103.8–104.3	106.6–107.0	110.3–110.8
5	149.2–150.9	139.0–140.8	131.0–132.6	100.8–103.6	104.3–105.9	108.3–110.2
10	146.5–149.8	136.7–139.5	129.0–131.8	98.2–102.2	102.2–104.6	106.2–109.3
15	143.8–148.8	133.8–137.8	126.9–130.8	95.8–100.6	100.1–103.1	104.0–108.0
20	141.2–147.5	131.0–135.9	124.8–129.7	94.0– 98.2	98.2–101.3	102.2–106.0
25	138.5–145.8	128.2–133.7	122.7–128.4	93.2– 93.6	96.8– 98.8	101.2–103.6
30	135.7–144.1	125.4–131.0	120.5–126.8	95.2–102.3	96.7– 98.2	100.8–101.6
35	132.5–142.2	122.6–128.4	118.4–124.9	98.4–107.0	99.1–102.5	103.0–105.2
40	129.2–139.8	119.8–125.5	116.3–122.7	102.1–111.1	102.2–106.2	105.2–108.5
45	124.8–137.2	116.8–122.7	113.7–120.0	105.8–114.8	105.2–109.7	107.5–111.6
50	120.5–134.0	113.8–119.7	110.9–117.0	109.3–118.2	108.1–112.9	109.7–114.3
55	115.6–130.3	110.8–116.7	108.0–113.8	112.9–121.3	111.1–115.8	111.8–116.3
60	110.7–124.8	107.7–113.0	104.7–109.9	116.0–124.4	114.0–118.6	114.1–118.2
65	105.6–118.3	104.4–108.7	101.8–105.6	119.2–127.5	116.7–121.2	116.1–119.8
70	100.7–111.1	100.7–103.3	99.0–100.4	122.4–130.2	119.3–123.7	118.2–121.3
75	97.1–103.3	96.7– 97.7	100.2–102.5	125.5–132.7	121.8–126.0	120.0–122.8
80	94.5– 96.1	98.4–100.7	101.8–104.4	128.5–135.1	124.2–128.0	121.9–124.2
85	95.9– 97.0	100.1–102.5	103.5–106.2	131.5–137.2	126.4–129.8	123.8–125.7
90	98.4– 99.9	102.1–104.1	105.4–108.0	134.8–139.0	128.6–131.3	125.7–127.0
95	101.1–102.3	104.2–105.6	107.6–109.5	138.1–140.5	130.8–132.4	127.4–128.2
100	103.8–104.3	106.6–107.0	110.3–110.8	141.4–141.9	132.8–133.1	128.7–129.0

and (*d*) adjacent acids, with the even-carbon one higher. The data obtained are presented graphically in Figures 1 to 4, in which the vertical lines between the curves represent the actual determinations. Melting ranges taken from the graphs at composition intervals of 5% by weight are given in Tables II and III. Approximate eutectic compositions and temperatures from the graphs are collected in Table IV.

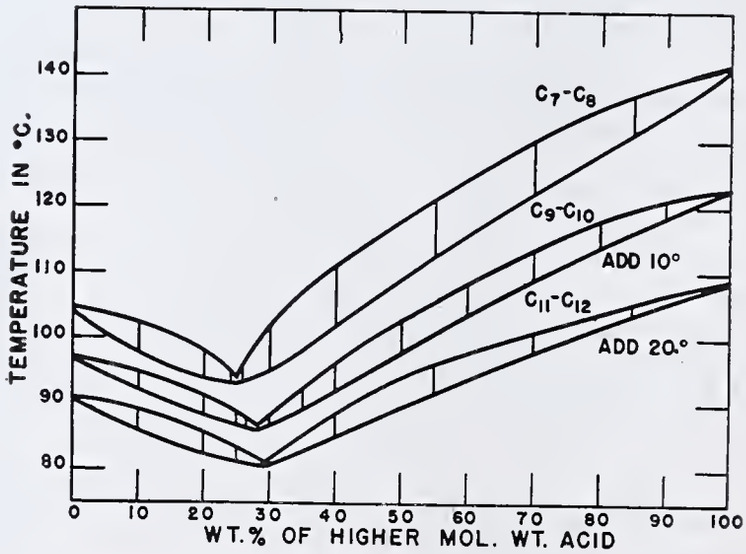

Figure 4. Composition–Melting Point Curves for Binary Mixtures of Adjacent Dibasic Acids with Higher Molecular Weight Even-Carbon Acid

Temperature scale to be shifted as indicated for individual curves

Table IV. Eutectic Temperatures in Binary Mixtures of Dibasic Acids

Binary System	Eutectic Temperature: By transition during heating	Eutectic Temperature: By graphical estimation	Weight % of Higher Molecular-Weight Acid in Eutectic Mixture
6–8	118–120	119.3–119.7	61
8–10	105–107.5	112.8–113.5	57
10–12	106–108.5	111.6–112.2	53
7–9	78.5–80.5	80.7–81.0	48.5
9–11	82–84.5	88.0–88.5	48
6–7	91.5–94	94.3–94.7	81
8–9	94–95	96.7–97.7	75
10–11	95–98.5	99.0–100.4	70
7–8	91–93	93.2–93.6	25
9–10	92–94.5	96.1–96.8	28
11–12	97.5–100	100.6–101.2	29.5

The eutectic temperatures fall int three groups: (*a*) below 90° for tw odd-carbon acids; (*b*) above 110° fc two even-carbon acids; and (*c*) bc tween 90° and 105° for adjacent acid The positions of the eutectics occu on the side of the lower-melting com ponent, and tend to approach a cer tral location as the acids become mor nearly alike.

REPRODUCIBILITY OF THE DATA

Melting ranges determined by single observer on successive sample usually check to ±0.1–0.2° C., and ar frequently within 0.1° C. for the uppe limit. Three different observers hav consistently agreed to ±0.2–0.3° C for the lower and ±0.1–0.2° C. for th higher temperature. An occasional sam ple behaved erratically, but this effect was readily overcome b determinations on duplicate samples. Duplicability of the value given in Tables II and III may be considered as ±0.25° C. o better when the sample size previously indicated is used, and th rate of heating through the melting range is not greater tha 0.2° per minute.

EVALUATION OF COMPOSITION FROM MELTING RANGE

The precision with which the composition of a mixture of know components may be determined depends on its relative positio in the binary system. Reproducibility of ±0.25° C. in the melt ing range values corresponds to less than 2.5% by weight fo most compositions, less than 1% for the most favorable propor tions, and as much as 5% in the region of single components o the flat minima of the even-even systems.

Distinction may be readily made between mixtures of simila melting ranges lying on opposite branches of the curve in a know binary system. When the components of a mixture are not defi nitely known, the possibilities will generally be limited to a very few by the previous history of the material. The approximat eutectic temperature may aid in classifying the mixture, for the two types of alternate-acid systems are usually distinguishable from each other and from those comprised of adjacent acids. Additional evidence obtained from the refractive index (*3*) and saponification equivalent of the methyl esters or neutralization equivalent of the acids will in most cases permit recognition of the components and composition of the mixture. Corroboration may be had by redetermination of the melting range after addition of a significant proportion of one of the pure components.

ACKNOWLEDGMENT

The writers acknowledge the assistance of F. T. Jones, who made the microscopical examination of representative acid mixtures, and of Julia S. Furlow, who checked many of the melting points and eutectic temperatures.

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Use of Refractive Indices of Dimethyl Esters

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easurements on purified dimethyl esters of dibasic acids having m six to twelve carbon atoms show that their refractive indices bear ear relations to change in temperature and to weight percentage the composition of binary mixtures. A change of 0.0001 index it corresponds to 3 to 6% change in composition of the mixtures, is approaching by a simple and rapid measurement the accuracy tainable by saponification equivalent determinations.

MEASUREMENT of refractive index presents a convenient and rapid means for following the progress of a fractional stillation, and extends the information furnished by the observed distillation temperatures. When careful fractionation elds only single components and intermediate binary mixtures, close estimate of composition may be obtained from a knowlge of the composition-index relations of binary mixtures. his procedure is nicely applicable in the analysis of dibasic ids. Distillation of the dimethyl esters is one of the most tisfactory ways of separating mixtures of the closely related ids, and the approximate compositions of the individual fracons can be readily determined through refractive index measements, as is shown in this paper. Further corroboration is ssible by determination of the melting ranges (*3*) of the revered acids.

PREPARATION OF METHYL ESTERS

All esters were prepared from the acids reported in the preding paper (*3*) except the C_{12} ester, which was fractionally ystallized from an original methyl ester distillation fraction. roperties of the esters are presented in Table I.

Table I. Properties of Esters Used in This Investigation

No. of Carbon Atoms in Acid	Boiling Point of Ester, ° C.	Melting Point of Ester, ° C.	
		This work	Literature values
6	71.4– 72.0 (0.2 mm.)	...	...
8	114.0–114.4 (4.7 mm.)	...	...
9	128.0–128.7 (5.2 mm.)	...	...
10	141.9–142.2 (6.0 mm.)	26	26.4 (*4*)
11	123.0–124.5 (2 mm.)	17	20.3 (*7*)
12	...	30.9–31.3	31 (*2*)

MEASUREMENT OF REFRACTIVE INDEX

Measurement of refractive index was performed with an Abbe-ype refractometer which could be read to 0.0001 index unit. 'emperatures reported for index readings were controlled to =0.05° C. by rapid circulation of water from a constant-temerature bath through the prism jackets of the refractometer. ll determinations were made on esters which had been freshly istilled or stored under reduced pressure to avoid errors caused y dissolved gases.

TEMPERATURE-INDEX RELATIONSHIPS

Indices were measured at 5° intervals in the range from 25° to 0° C. for all temperatures at which the esters were liquid. The xpected straight-line relationships were found, and are expressed by the following equations, in which t represents degrees Centigrade:

Dimethyl adipate	$n_D^t = 1.4205 - 0.00040_5(t - 40)$
Dimethyl suberate	$n_D^t = 1.4262 - 0.00039_5(t - 40)$
Dimethyl azelate	$n_D^t = 1.4284 - 0.00039_5(t - 40)$
Dimethyl sebacate	$n_D^t = 1.4307 - 0.00039(t - 40)$
Dimethyl 1,11-undecanedioate	$n_D^t = 1.4329 - 0.00038_5(t - 40)$
Dimethyl 1,12-dodecanedioate	$n_D^t = 1.4345 - 0.00038(t - 40)$

The index values are in agreement with those given by Karvonen (*4*), and the temperature increments are of the same magnitudes as those found by Mattil and Longenecker (*5*) for monobasic methyl esters and for synthetic glycerides.

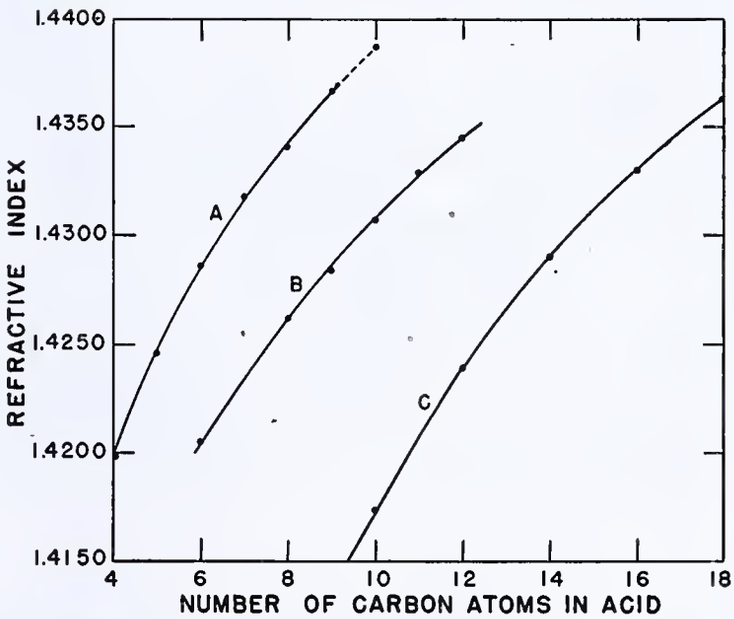

Figure 1. Refractive Indices of Methyl Esters of Normal Monobasic and Dibasic Aliphatic Acids

A. $N_D^{20.0}$ of dimethyl esters of dibasic acids, from Karvonen (*4*)
B. $N_D^{40.0}$ of dimethyl esters of dibasic acids, this investigation
C. $N_D^{40.0}$ of methyl esters of monobasic acids, from Mattil and Longenecker (*5*)

For any single temperature the indices of the homologous esters are represented by a smooth curve as shown in Figure 1. Corresponding indices for monobasic methyl esters (*5*) are included for comparison, as are those of Karvonen for the dibasic esters at 20° C. (value for methyl sebacate is calculated from 28° C. by the equation presented above).

COMPOSITION-INDEX RELATIONSHIP IN BINARY MIXTURES

Several series of binary mixtures were prepared which represented various possible types, and their refractive indices were measured at 40° C. The composition-index relation could be represented by a straight line in all cases when the composition was expressed as weight percentages, as shown in Figure 2. The relation was nonlinear for molecular percentages. Here again the results are in accord with those of Mattil and Longenecker for monobasic methyl esters.

As the difference in indices of adjacent homologous esters varies from about 0.0030 between C_6 and C_7 ($n_D^{40.0}$ for dimethyl

[1] Second article in series; first appears on page 538.

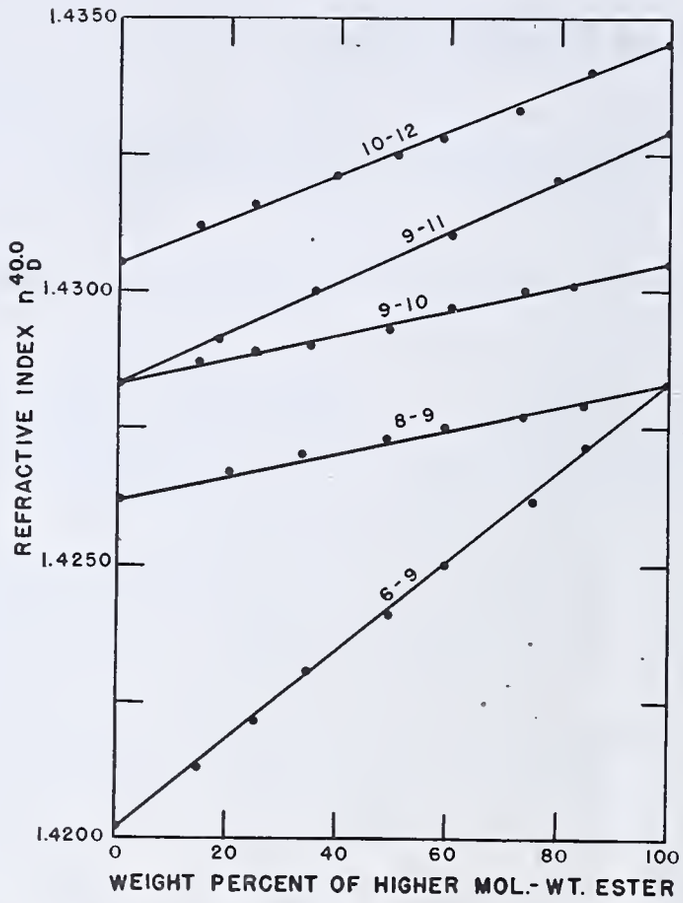

Figure 2. Refractive Indices of Binary Mixtures of Dimethyl Esters of Dibasic Acids at 40° C.

Numbers designate carbon content of component acids

pimelate estimated graphically as 1.4235) to 0.0016 between C_{11} and C_{12}, a difference of 0.0001 in refractive index corresponds to a 3 to 6% change in composition in binary mixtures of adjacent esters. This is about the same precision obtainable from equivalent weight determinations in which a difference of 0.2 unit corresponds to approximately 3%.

INTERFERENCES

One possible source of error is the presence of dissolved gases If the liquid dimethyl esters are exposed to the atmosphere, th refractive index will drop several fourth-place units over a perio of a day or so. Original values can be obtained following re distillation or degassing, but are not achieved by the use o desiccants. This type of error can be avoided readily by makin index determinations promptly after distillations, or by storin the liquid esters at reduced pressures.

Methyl esters of monobasic acids such as myristic or palmiti may be present in products from oxidative cleavage (*1*) of un saturated acids from various sources, although they woul normally be removed by partitions prior to analysis of the dibasi acids. If any remained, they would interfere with the dete mination of composition by means of refractive index. Meth myristate, for example, distills (*6*) in the temperature rang between dimethyl sebacate and undecanedioate, but has refractive index almost as low as dimethyl azelate (Figure 1 In such a case the calculated composition would indicate to large a proportion of the lower molecular-weight component However, the apparent composition derived from determinatio of the saponification equivalent would be in error in the opposit direction. Accordingly, agreement between the results obtaine by the two methods would confirm the absence of monobasi esters and enhance the validity of the analysis.

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Volumetric Determination of Magnesium in Magnesium Carbonate Ores

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IN DETERMINING the magnesium content of carbonates, many investigators have used a measured excess of standard alkali to precipitate magnesium hydroxide and have determined the excess with a standard acid. The outstanding difference in the methods is in the indicators used: Kolthoff (*3*) used phenolphthalein and dimethyl yellow; Pierce and Geiger (*5*) used trinitrobenzene and bromophenol blue; and Willstätter and Waldschmidt-Leitz (*7*) used thymolphthalein. Most of the methods employed more than one indicator and specified the use of carbonate-free standard alkali solution. In many investigations the emphasis seemed to be on the saving of time rather than a high degree of accuracy.

The chief objectives of this investigation were (1) to develop an accurate routine method for the volumetric determination of magnesium that could be carried out in a reasonable length of time and would not require carbonate-free standard alkali, and (2) to find a single indicator that could be used for all the neutralizations required in the procedure.

THEORETICAL

In selecting a single indicator for this investigation two condi tions had to be satisfied: The iron and aluminum, which are th most common impurities found in carbonate ores, should be com pletely precipitated as the insoluble hydroxides at the end point and the indicator should show the true equivalence point whe standard alkali which contains carbonates is neutralized b standard acids.

Blum (*1*) states that the precipitation of aluminum hydroxid begins at pH 3 and is complete at or before pH 7 and recommend the use of methyl red as the indicator. Since ferric hydroxide i completely precipitated under the same conditions, aluminur and iron can be removed quantitatively at the methyl red en point. There should be no postprecipitation of magnesiur hydroxide if the neutralization is carefully carried out.

At the methyl orange end point (pH 4) the absorption of carbo dioxide shows no decrease in the effective normality of a standar alkali solution (*6*); therefore, methyl red (pH 4.2 to 6.3) shoul

Table I. Effect of Calcium

	Gravimetric Pyrophosphate Method, Prepared MgO	Proposed Volumetric Method Prepared MgO	Proposed Volumetric Method Prepared MgO + $CaCO_3$
	%	%	%
	98.15	98.36	98.36
	97.93	98.05	98.36
	98.44	98.36	98.05
	98.67	98.36	98.05
	...	98.05	98.05
	...	98.36	98.36
	...	98.05	98.36
	...	98.05	98.36
Av.	98.30	98.20	98.24

show the true equivalence point when 0.5 *N* sodium hydroxide which contains carbonate is titrated with a standard acid.

Manganese, which is often found as an impurity in carbonate ores, is not precipitated as manganous hydroxide at the methyl red end point and could be oxidized to insoluble manganese dioxide by sodium peroxide in alkaline solution.

Time could be saved by decomposing and fuming the ore with sulfuric acid. This would dehydrate the silica and remove excess calcium as insoluble calcium sulfate.

EXPERIMENTAL

Using methyl red as the indicator, the acid titer of 0.5 *N* carbonate-free sodium hydroxide solution was determined before and after air was drawn through the alkali for 10 hours. There was no appreciable change in the titer, indicating that methyl red would show the true equivalence point when carbonates are present in 0.5 *N* sodium hydroxide solution. A double indicator titration (phenolphthalein and methyl red) showed that 0.4 mg. of carbon dioxide had been absorbed per milliliter.

To determine the accuracy of the proposed method the purity of some prepared calcium-free magnesium oxide was determined. The oxide was prepared by igniting magnesium nitrate, C.P., Baker's analyzed, which had been recrystallized from water five times. No calcium was found in the final product.

The purity of the prepared magnesium oxide was determined gravimetrically by the conventional pyrophosphate method (*2*) and volumetrically by the proposed procedure, using 0.200-gram samples. To study the effect of calcium the volumetric determinations were repeated, using mixtures of 0.200 gram of prepared magnesium oxide and 0.200 gram of precipitated calcium carbonate (Table I).

The method was then used on Bureau of Standards samples 1A, 88, and 104, and three limestone samples from Thorn Smith, Detroit, Mich. Since Bureau of Standards sample 104, burned magnesite, showed a high magnesium oxide content, a 0.100-gram sample was used. The results of these determinations are recorded in Table II.

SPECIAL REAGENTS

Sodium hydroxide solution, 0.50 *N* standardized by using sulfamic acid, LaMotte, as the primary standard (*6*) and 1% methyl red solution as the indicator.

Sulfuric acid solution, 0.25 *N* standardized using 0.50 *N* sodium hydroxide solution.

Methyl red indicator solution (*4*), 1%.

PROCEDURE

Weigh into a beaker 0.500 gram of the carbonate ore and cover it with water. Slowly add 10 ml. of 6 *N* sulfuric acid, boil until the ore is decomposed, and carefully evaporate until all the sulfuric acid has been expelled. Take up the residue in about 25 ml. of hot water, boil for 2 or 3 minutes to ensure complete solution of all soluble salts, and filter, washing the residue four or five times with hot water. This residue is composed of acid-insoluble substances such as silica and calcium sulfate. Dilute the filtrate to about 100 ml., neutralize with 6 *N* sodium hydroxide, and add about 0.5 gram of sodium peroxide with stirring. Boil for about 20 minutes or until the excess sodium peroxide is removed. (If manganese is not present in the ore, the peroxide treatment may be omitted.) Acidify with 6 *N* sulfuric acid and boil for a few minutes to ensure the complete solution of all salts. Add 3 or 4 drops of 1% methyl red indicator solution and carefully adjust the acidity to the methyl red end point, using 0.50 *N* sodium hydroxide and 0.25 *N* sulfuric acid. Boil for a few minutes and filter into a 250-ml. volumetric flask, washing the residue of insoluble hydroxides four or five times with hot water. Just acidify the filtrate with 0.25 *N* sulfuric acid and add exactly 25 ml. of 0.50 *N* sodium hydroxide. After thorough mixing, cool the contents of the flask to room temperature, dilute to exactly 250 ml., and pour into a large beaker.

After the precipitate of magnesium hydroxide has settled (20 to 30 minutes), decant the supernatant liquid through a quantitative filter into a 250-ml. volumetric flask containing 50 ml. of distilled water until the total volume is exactly 250 ml. Transfer the solution to a large beaker, add one drop of methyl red solution, and titrate with 0.25 *N* sulfuric acid to the end point. The titration can be speeded up by adding a drop of phenolphthalein solution before the final titration is made. At the phenolphthalein end point the color change is from red to yellow, occurring 10 to 15 drops from the methyl red end point.

DISCUSSION

The results in Table I show that the volumetric values are consistent and the average is in good agreement with that obtained for the pyrophosphate gravimetric determinations. The maximum difference was 0.74% in the four pyrophosphate determinations and 0.31% in the eight volumetric determinations. Appreciable amounts of calcium had no effect on the determinations.

The values for the ore determinations also show good consistency and the averages are comparable with the certified magnesium oxide values.

Table II. Determination of Magnesium Oxide in Carbonate Ores

	Bureau of Standards			Thorn Smith		
	1A (2.19%)	88 (21.48%)	104 (85.67%)	1 (11.64%)	2 (13.27%)	3 (14.14%)
	%	%	%	%	%	%
1	2.19	21.38	85.00	11.75	13.31	14.25
2	2.19	21.44	85.62	11.69	13.31	14.19
3	2.25	21.38	85.31	11.63	13.31	14.25
4	2.25	21.44	85.00	11.63	13.37	14.13
5	2.19	21.50	85.31	...	...	...
6	2.25	21.38	85.31	...	...	...
7	2.25	21.38	85.31	...	...	...
8	2.19	21.50	85.62	...	...	...
9	2.25	21.50	85.31	...	...	...
10	2.25	21.38	85.31	...	...	...
Av.	2.23	21.43	85.31	11.68	13.32	14.20

The differences can be attributed to unavoidable experimental error, for one drop of 0.25 *N* sulfuric acid corresponds to about 0.06% of magnesium oxide for a 0.500-gram sample of ore and about 0.31% for a 0.100-gram sample. The maximum difference in the Bureau of Standards determinations on the three ores was 0.29% for sample 104. The volumetric determinations showed a maximum difference of 0.62% for the same ore, but 0.100-gram samples were used which represented a difference of only 2 drops of 0.25 *N* sulfuric acid in obtaining the final end points.

Methyl red is the single indicator that is used for all the neutralizations throughout the procedure. As it shows the true equivalence point in the presence of carbonates, it is not necessary to use carbonate-free standard alkali for the determinations. Common impurities such as iron and aluminum are completely removed as the insoluble hydroxides at the methyl red end point.

Manganese can be removed by oxidizing it to the insoluble dioxide with sodium peroxide in alkaline solution.

The determination can be carried out in less than 4 hours, and the time is shortened if manganese is not present, since the peroxide treatment is not necessary. If the precipitate of magnesium hydroxide is small, the final filtration can be carried out without allowing the precipitate to settle, thus speeding up the determination. For samples of high magnesium content the same result can be accomplished by using samples smaller than 0.500 gram or by using aliquots of the solution obtained from a 0.500-gram sample; however, accuracy is sacrificed for speed. Time is also saved by using sulfuric acid to decompose the ore and dehydrate the silica.

SUMMARY

The proposed volumetric method for the determination of magnesium in carbonate ores involves removal of most of the calcium as the sulfate, precipitation of magnesium hydroxide with excess standard alkali, and titration of the excess with a standard acid. When methyl red is used as the indicator, 0.50 *N* sodium hydroxide solution that is not carbonate-free can be satisfactorily used. Any indicator having the same pH range as methyl red could be used, but methyl red was selected because it is used in many laboratories as an acid-base indicator. Common impurities such as iron and aluminum are removed as insoluble hydroxides at the methyl red end point and manganese can be removed by oxidizing it to insoluble manganese dioxide with alkali peroxide.

This method gives results that are comparable with those obtained with the conventional gravimetric pyrophosphate method. It does not require as much time as the pyrophosphate method and should be useful for the routine analysis of magnesium carbonate ores.

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Analysis of Oil-Soluble Petroleum Sulfonates

Extraction-Adsorption Method

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A relatively simple extraction-adsorption method for the analysis of oil-soluble petroleum sulfonates is described. The method is applicable to the analysis of sodium and other metal sulfonates and is free of lengthy operations and emulsion difficulties. Procedures are included for the determination of mineral oil, sulfonic acid soap, water, carboxylic acid soap, free alkali, and inorganic salts.

PETROLEUM sulfonic acids are produced by the treatment of petroleum distillates with sulfuric acid in refining processes or by the treatment of petroleum stocks with oleum in the manufacture of medicinal white oils. Some of the sulfonic acids formed remain in the oil layer, and the others are found dissolved in the acid layer. The former type is frequently referred to as "mahogany" acids, while the latter is termed "green" acids. Following the sulfonation process, the "acid oil" layer is separated from the sludge containing the water-soluble green acids and neutralized with caustic, and the crude mahogany soaps are extracted with dilute alcohol.

The crude mahogany soaps obtained in this manner consist principally of approximately equal quantities of sulfonic acid soaps and mineral oil, with minor amounts of water, carboxylic acid soaps, free alkali, and inorganic salts. For the purpose of evaluating these mixtures it is desirable to determine their compositions, particularly the sodium sulfonate contents. Archibald and Baldeschwieler (*2*) have described a procedure of analysis involving a rather tedious extraction of the mineral oil with petroleum ether from a suspension of the sample in 50% aqueous isopropyl alcohol. This procedure is very lengthy and does not include a method for determining carboxylic acid soap content. More recently, an adsorption method for separating and estimating the constituents has been described by Koch (*3*). This method involves separation of the sulfonate soaps from the mineral oil by passing a petroleum ether solution of the material through a column of Attapulgus clay. The mineral oil and petroleum ether pass through the clay, and the solution is collected, the petroleum ether evaporated, and the mineral oil weighed. The sulfonate soap is eluted from the clay with methanol and the weight obtained by evaporating the methanol from the resulting solution. This is very rapid but is not satisfactory when applied to materials containing appreciable quantities of water or inorganic salts. Experience in this laboratory has shown that the elution of the sulfonate soaps with methanol is incomplete, leading to low values for this component.

During 1944 an extensive program of cooperative testing of methods for the analysis of petroleum sulfonates was conducted by the A.S.T.M., as a result of which, a method has been proposed (*1*) which covers the determination of mineral oil, sodium sulfonate, carboxylate soap, inorganic salt, and free alkali contents, combining weight and specific gravity. The method involves multiple extractions of an aqueous isopropyl alcohol suspension of the sample with petroleum ether. The mineral oil passes into the petroleum ether phase and is thus isolated. The alcohol phase is then subjected to a number of rather lengthy manipulations to separate the sodium sulfonates and carboxylates from the remaining constituents. Although the method yields fairly accurate and precise data, the manipulations involved are both tedious and time-consuming; a complete analysis requires about 4 days.

In view of the recognized shortcomings of the A.S.T.M. and Archibald-Baldeschwieler methods with regard to time consumption, and Koch's adsorption method with regard to inapplicability in the presence of inorganic salts and water as well as incomplete recovery of the adsorbed sulfonates, an attempt has been made by the authors to devise a method of analysis which is free of these objections.

This paper presents a new method in which the sulfonic acids, carboxylic acids, and mineral oil are separated from the inorganic salts by extracting an acidified aqueous suspension of the sample with chloroform. The inorganic salt content is calculated from the sulfated residue obtained from the aqueous phase, making correction for the alkali residue contributed by the sulfonate, carboxylate, and free alkali. A portion of the residue obtained from the chloroform extract is titrated potentiometrically for strong and weak acids to determine the respective equivalents of sulfonic and carboxylic acids present. Another portion of the chloroform-soluble residue is neutralized and the mineral oil isolated by adsorbing the sulfonates and carboxylates on Attapulgus clay. A schematic diagram of this method of analysis is shown in Figure 1.

APPARATUS AND MATERIALS

The percolation apparatus of Koch has been modified somewhat to permit the use of pressure and subsequently a more rapid rate of flow through the adsorbent.

The modified percolator is shown in Figure 2. It was found that a pressure of 351 to 703 kg. per square meter (0.5 to 1 pound per square inch) forced the liquid through sufficiently rapidly. The adsorbent used was the same as that recommended by Koch (30- to 60-mesh Attapulgus clay which has been calcined at 487° C., obtained from the Attapulgus Clay Company, 260 South Broad St., Philadelphia, Pa.).

Inverted-rim beakers of the type described by the A.S.T.M. (*1*) were used for evaporations where there was danger of loss due to "creeping" over the sides.

The chloroform and petroleum ether used were of C.P. quality.

A Precision-Shell (*4*) titrometer was used for the electrometric titration of strong and weak acids.

PROCEDURE

Separation of Organic and Inorganic Constituents. Introduce approximately 10 grams of sample weighed to the nearest centigram into a 100-ml. beaker, add 25 ml. of chloroform, and stir to dissolve or disperse the sample.

Transfer quantitatively to a 250-ml. separatory funnel, rinsing the beaker and stirring rod with an additional 25 ml. of chloroform. Rinse the sampling beaker with 50 ml. of 6 *N* hydrochloric acid and add this solution to the separatory funnel. Shake the separatory funnel and contents vigorously, venting frequently. Rinse the sampling beaker with 50 ml. of acetone and add this to the separatory funnel. Mix intimately once more and allow the two phases to separate. Draw the chloroform extract (lower phase) into a weighed, inverted-rim beaker. Extract the aqueous phase three more times with 25-ml. portions of chloroform and combine the chloroform extracts in the inverted-rim beaker.

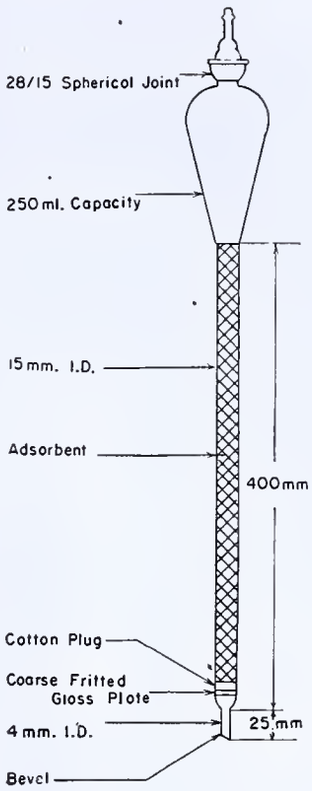

Figure 2. Special Pressure Adsorption Column

Evaporate the combined chloroform extracts to dryness on a steam bath, heating for 15 minutes after the disappearance of the odor of chloroform. Cool to room temperature and weigh the beaker and contents.

Determination of Free Salts. Draw the aqueous layer obtained above into a weighed, ignited platinum dish and evaporate to dryness on a steam bath. Add approximately 2 ml. of 36 *N* sulfuric acid and rotate the dish in such a manner as to moisten all the residue with the acid. Heat carefully (to prevent spitting) over a small flame until fuming ceases. Repeat this treatment with 1 ml. of sulfuric acid. When fuming ceases, heat in a muffle furnace at 800° ± 50° C. to constant weight.

Determination of Sulfonate and Carboxylate Soaps. Determine the number of equivalents of strong and weak acids present in 100 grams of the chloroform extract by electrometric titration (A.S.T.M. D664–44T) of an accurately weighed 1- to 2-gram sample. Also analyze this residue for water content by the Karl Fischer method. (Place 20 ml. of anhydrous pyridine and 10 ml. of anhydrous ethyl ether in a 100-ml. glass-stoppered volumetric flask and titrate to the red-brown Fischer end point. Weigh 0.5 to 1 gram of sample in a glass weighing boat, slide the boat into the flask, and swirl until the sample is completely dissolved. Titrate the solution with Fischer reagent to the original red-brown end point. For the preparation and standardization of the Fischer reagent, see A.S.T.M. procedure D268–44T, issued under the jurisdiction of Committee D-1.)

Determination of Mineral Oil. Weigh approximately 2 grams of the chloroform extract to the nearest milligram into a 100-ml. beaker. Add sufficient 0.5 *N* alcoholic sodium hydroxide solution to neutralize completely the strong and weak acids present (this volume is readily calculated from the data obtained by titration above), then add 0.1 ml. in excess. Evaporate the alcohol on a steam bath. Add 25 ml. of petroleum ether and stir to dissolve the sample completely. Transfer this solution quantitatively to the 250-ml. reservoir of the percolation apparatus (Figure 2), rinsing the beaker and stirring rod with an additional 25 ml. of petroleum ether. Apply a pressure of 0.5 to 1 pound per square inch at the top of the percolator and force the solution through the 40-cm. column of adsorbent clay. Force an additional 150 ml. of petroleum ether through the column in similar fashion. Collect the solution which passes through the clay in a weighed, inverted-rim beaker and evaporate to dryness on a steam bath, heating for 15 minutes after disappearance of the odor of petroleum ether. Heat for 15-minute intervals at 110° C. until the weight loss between successive heatings is less than 1 mg.

Determination of Water. Determine the water content of the original sample by A.S.T.M. Method D95–40 or, if the free alkali content is low, by the Karl Fischer method.

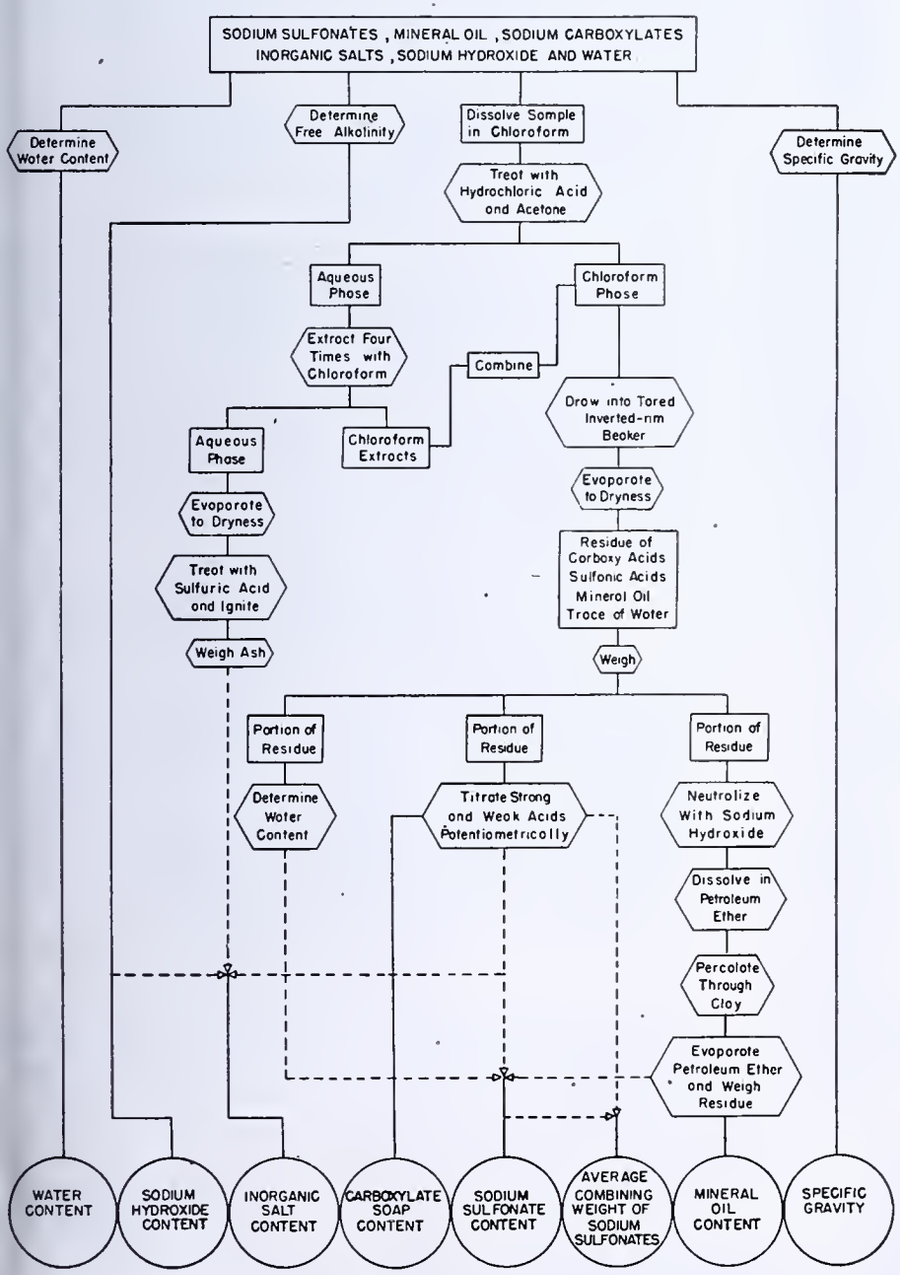

Figure 1. Schematic Diagram of Analysis of Petroleum Sulfonate Mixtures

Table I. Comparison of Analyses by Extraction-Adsorption and A.S.T.M. Methods

Constituent	Sample A				Sample B			Sample C			Sample D		Sample E	
	Extraction-Adsorption Method			A.S.T.M. method	Extraction-Adsorption Method		A.S.T.M. method	Extraction-Adsorption Method		A.S.T.M. method	Extraction-adsorption method	A.S.T.M. method	Extraction-adsorption method	A.S.T.M. method
	Run 1	Run 2	Run 3	Av.	Run 1	Run 2	Av.	Run 1	Run 2	Av.				
Mineral oil, %	42.6	42.0	43.0	42.5	36.4	36.8	36.6	36.1	36.2	37.2	36.7	36.2	53.6	53.2	54.8	55.0
Sodium sulfonate, %	46.0	45.8	45.5	46.0	54.6	55.8	55.2	55.1	50.6	49.9	50.2	50.7	36.8	37.4	32.2	32.0
Sodium carboxylate, %	0.8	1.1	1.2	1.0	1.0	0.7	0.8	0.2	1.5	1.0	1.2	0.5	3.0	2.4	0.6	0.8
Sodium hydroxide, %	0.0	0.0	0.0	0.0	0.2	0.2	0.2	0.2	0.3	0.3	0.3	0.3	0.0	0.0	0.9	0.9
Water, %	4.0	4.0	4.0	4.0	6.1	6.1	6.1	6.1	7.8	7.8	7.8	7.8	5.3	5.3	7.0	7.0
Inorganic salts, %	7.0	6.8	7.0	7.3	1.6	1.4	1.5	1.3	3.4	3.6	3.5	4.4	2.4	2.5	4.0	4.5
Total, %	100.4	99.7	100.7	100.5	99.9	101.0	100.4	99.0	99.8	99.8	99.8	99.9	101.1	100.8	99.5	100.2
Combining weight of sodium sulfonate	487	474	471	477	571	531	551	542	560	543	552	566	473	493	413	416

DETERMINATION OF FREE ALKALI. Weigh approximately 10 grams of the original sample into a 250-ml. Erlenmeyer flask, add 50 ml. of ethyl alcohol and 50 ml. of benzene, and swirl until dissolved. Add 6 or 7 drops of thymolphthalein indicator solution and titrate with standard 0.5 N sulfuric acid solution until the color matches that of a standard prepared in the same manner except for the addition of indicator. This is essentially the method recommended by the A.S.T.M. (*1*).

DETERMINATION OF SPECIFIC GRAVITY. Fill the pycnometer (25-ml. Weld-type pycnometer fitted with a special stopper having a 4- to 5-mm. diameter bore) with the original sample, warming to 70° or 80° C. until all air bubbles have risen to the top. Cool to 15° to 20° C., adding more sample if necessary, so that the pycnometer remains full. Insert the stopper, pressing slowly into place with a turning motion. If the sample is too viscous to permit insertion of the stopper, carefully warm the neck of the pycnometer and the stopper with a small Bunsen burner. Place the pycnometer, with the cap in place, in a bath at 25° ± 0.1° C. After 25 minutes, remove the pycnometer, cool to 15° to 20° C., and remove any excess sample by wiping with a cloth moistened with a benzene-alcohol mixture. Replace the cap and weigh to the nearest milligram. Record the sample weight.

CALCULATION. Calculate the quantities of various components present in the sample by means of the following equations:

$$\text{Mineral oil content, per cent} = \frac{(A)(D)(100)}{(C)(W)}$$

$$\text{Inorganic salt content, per cent} = \frac{(100)(B) - (71)(A)(E + e)}{W} - \frac{(71)(G)}{40}$$

(The value 71 is the combining weight of sodium sulfate.)

$$\text{Sodium sulfonate content, per cent} = \frac{(A)(100 + 22E)}{W} - X - \frac{A}{W}[Y + (312)(e)]$$

(The numerical value 312 is the assumed average combining weight of the carboxylic acids. The value 22 converts from the strong acid to the sodium salt.)

$$\text{Carboxylate soap content, per cent} = \frac{(A)(e)(334)}{W}$$

(The value 334 is the assumed average combining weight of the carboxylate soaps.)

$$\text{Combining weight of sodium sulfonate} = \frac{(F)(W)}{(A)(E)}$$

$$\text{Free alkali content, as sodium hydroxide, per cent} = \frac{(V)(N)(4.0)}{W_1}$$

$$\text{Specific gravity } 25°/25° \text{ C.} = \frac{W_s}{W_c}$$

where

A = weight of chloroform-soluble organic residue, grams
C = weight of organic residue used for mineral oil determination
D = weight of mineral oil found
W = weight of original sample taken for analysis
B = weight of sulfate ash obtained from aqueous residue
E = strong acids, equivalents per 100 grams of organic residue
e = weak acids, equivalents per 100 grams of organic residue
G = free alkali content, per cent
X = mineral oil content of sample, per cent
Y = water content of organic residue, per cent
F = sodium sulfonate content, per cent
V = milliliters of sulfuric acid required to neutralize free alkali
N = normality of sulfuric acid
W_1 = weight of sample used in free alkali determination
W_s = weight of sample contained in pycnometer at 25° C.
W_c = weight of water contained in pycnometer in calibration

RESULTS

In Table I are shown the results of analyses of several crude petroleum sulfonate samples from various sources by both the extraction-adsorption and A.S.T.M. methods; the two methods yield very similar data. In general, the extraction-adsorption method gives slightly higher values for carboxylate soap content than the A.S.T.M. method. Both procedures determine this component by titrating the free carboxylic acid and calculate the carboxylate content on the basis of an assumed (by convention) combining weight of 334 for the soap. However, the titration carried out in the extraction-adsorption method is considerably more direct and hence is believed the more valid.

In Table II are shown the results obtained when the described method was applied to a synthetic petroleum sulfonate blend of known composition (sample furnished by Stanco, Inc., Elizabeth, N. J.). The data obtained compare favorably with the theoretical composition of the material. The values for sulfonate content are slightly lower than the known content, and the values for carboxylate content are high. However, the sum of carboxylate and sulfonate contents in both analyses compare favorably with the known sum of these two quantities. This indicates that for this particular sample the assumed combining weight of the carboxylate soap (334) is too high.

DISCUSSION

The method described differs fundamentally from other extraction methods in that the sulfonates are converted to sulfonic acids prior to extraction with a solvent. This is generally an advantage, because sulfonates of metals other than sodium may thus also be analyzed. Such metallic sulfonates do not alter the chloroform extraction of the free acids but undoubtedly influence the results of any method which involves a direct extraction of the soaps, in that the distribution ratios between the solvents may vary considerably. Satisfactory results have been obtained by the extraction-adsorption method when applied to the analysis of barium and calcium sulfonates.

Table II. Analysis of Synthetic Sulfonate Sample[a] by the Extraction-Adsorption Method

Constituent	Data Obtained Run 1	Run 2	Av.	Known Composition
Mineral oil, %	50.1	50.1	50.1	49.6
Sodium sulfonate, %	39.5	39.1	39.3	40.6
Sodium carboxylate, %	4.3	4.3	4.3	3.0
Sum of sulfonate and carboxylate, %	(43.8)	(43.4)	(43.6)	(43.6)
Sodium hydroxide, %	0.0	0.0	0.0	0.0
Water, %	3.9	3.9	3.9	3.6
Inorganic salts, %	3.3	3.2	3.2	3.1
Total analysis, %	101.0	100.6	100.8	99.9
Combining weight of sodium sulfonate	465	456	461	...

[a] Sample furnished by Stanco, Inc., Elizabeth, N. J.

Considerable difficulty with emulsion formation has resulted when other extraction methods have been used in analyzing petroleum sulfonate mixtures. The function of the acetone in the method described is to break the emulsion which forms between the chloroform and dilute acid layers. Isopropyl alcohol was tried first as a demulsifying agent and although it broke the emulsions quickly, the results were erroneous, owing to formation of sulfonic acid esters with the alcohol. For this reason a demulsifier was sought which was unreactive toward the free sulfonic acid and at the same time sufficiently volatile to be readily evaporated on a steam bath. Acetone fits these requirements very well; it has proved very satisfactory and in all instances thus far has resulted in instant and complete separation of the aqueous and chloroform phases.

By employing the chloroform extraction procedure, an analysis may be completed in 2 days' elapsed time. This is considerably less than the 4 days required in this laboratory for an analysis by the A.S.T.M. method, and there are fewer manipulations (weighings, extractions, etc.) than in the A.S.T.M. method, so the handling time per determination is appreciably less. In control analyses, where the combining weight of the sodium sulfonate is known from previous experience, the method may be further shortened by eliminating the adsorption step, calculating the sulfonate content directly from the strong acid titration of the chloroform-soluble residue, and obtaining the mineral oil content by difference. This is a distinct advantage over other extraction methods, which permit no such short-cut modifications.

The method described is inapplicable, without sacrificing accuracy, to the analysis of materials containing volatile oils. The composition of samples of this type can be approximated by application of the procedure described, elution of the sulfonates from the Attapulgus clay with methanol to recover the adsorbed sulfonates and carboxylates, and evaporation of the methanol to obtain the weight of sulfonate and carboxylate. The mineral oil content is then determined by difference. Generally this procedure leads to sulfonate values low by 0.5 to 2%, and mineral oil values high by a corresponding amount, owing to incomplete recovery of sulfonate from the clay.

Although chloroform extraction can be applied to green acid soaps as well as mahogany acid soaps, the chloroform extract from green acid soaps is insoluble in petroleum ether and hence the sulfonates and carboxylates cannot be separated from mineral oil by the adsorption procedure described. For this reason attempts to apply the method to green acid sulfonates have been unsuccessful.

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Presented before the Division of Analytical and Micro Chemistry at the 109th Meeting of the American Chemical Society, Atlantic City, N. J.

Composition of Cellulose Esters

Use of Equations and Nomographs

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The composition of cellulose esters in terms of the number of ester groups per anhydroglucose unit may be determined readily from analytical data by calculations or by reading from nomographs. Equations and nomographs are given for cellulose acetates, propionates, butyrates, acetate propionates, and acetate butyrates. By either method the per cent acetyl and per cent propionyl or butyryl in any of these cellulose esters can be easily converted to the number of acetyl, propionyl, or butyryl, and hydroxyl groups per anhydroglucose unit or vice versa.

METHODS for the analysis of cellulose esters, such as those reported from this laboratory, give results in terms of per cent "apparent acetyl" (*2*, *6*), per cent acetyl, propionyl, or butyryl (*7*), and per cent hydroxyl (*5*). From the viewpoint of structural organic chemistry, however, it is desirable to express the composition of these esters in terms of the numbers of substituent groups per anhydroglucose unit. This information is required for certain calculations, such as that of unit molecular weight. Some equations for this purpose have been published by Gloor (*3*), by Malm, Genung, and Williams (*5*), and in a Navy specification (*8*). Simpler equations have been derived and are presented here for the cases of cellulose acetates, acetate propionates, and acetate butyrates.

Nomographs have been constructed by means of which this conversion from per cent acyl to number of groups per anhydroglucose unit of cellulose can be made very rapidly for the cases of cellulose acetates, propionates, butyrates, acetate propionates, and acetate butyrates.

EQUATIONS

The equations given below are based on the general formula for the anhydroglucose unit of a cellulose ester, such as an acetate propionate.

$$(C_6H_7O_5)\begin{cases} -(OCCH_3)N_a \\ -(OCC_2H_5)N_p \\ -H(3-N_a-N_p) \end{cases}$$

The following terminology is used:

a = weight per cent of acetyl
p = weight per cent of propionyl
b = weight per cent of butyryl

h = weight per cent of hydroxyl
N_a = number of acetyl groups per anhydroglucose unit
N_p = number of propionyl groups per anhydroglucose unit
N_b = number of butyryl groups per anhydroglucose unit
N_h = number of hydroxyl groups per anhydroglucose unit

and the unit molecular weight of a cellulose acetate propionate is

$$M.W. = 159 + 43N_a + 57N_p + 3 - N_a - N_p$$
$$= 162 + 42N_a + 56N_p \quad (1)$$

These calculations assume only three replaceable hydroxyls per anhydroglucose unit. This assumption is valid for most ordinary cellulose esters. It is not strictly true in cases of very degraded and low-viscosity celluloses and their derivatives because the number of hydroxyl groups on the ends of the chains may be significant.

Case of Cellulose Acetate. Weight per cent acetyl, which may be found by analysis, is 100 times the weight of an acetyl group, 43, times the number of such groups, and divided by the unit molecular weight:

$$a = \frac{4300N_a}{162 + 42N_a} \quad (2)$$

Solving Equation 2 for N_a gives

$$N_a = \frac{3.86a}{102.4 - a} \quad (3)$$

$$N_h = 3 - N_a \text{ by assumption} \quad (4)$$

$$h = \frac{1700(3 - N_a)}{162 + 42N_a} = \frac{1700N_h}{288 - 42N_h} \quad (5)$$

Weight per cent acetyl and weight per cent hydroxyl can be converted readily one to the other using the equations:

$$h = \frac{44.8 - a}{1.42} \quad (6)$$

$$a = 44.8 - 1.42h \quad (7)$$

which were derived from Equations 5 and 3.

Case of Cellulose Acetate Propionate. Equations for weight per cent acetyl and propionyl are as follows:

$$a = \frac{4300N_a}{162 + 42N_a + 56N_p} \quad (8)$$

$$p = \frac{5700N_p}{162 + 42N_a + 56N_p} \quad (9)$$

Solving Equations 8 and 9 simultaneously gives:

$$N_a = \frac{3.86a}{102.4 - 1.006p - a} \quad (10)$$

$$N_p = \frac{2.91p}{102.4 - 1.006p - a} \quad (11)$$

$$N_h = 3 - N_a - N_p \text{ by assumption} \quad (12)$$

$$h = \frac{1700N_h}{162 + 42N_a + 56N_p} \quad (13)$$

The corresponding equations for cellulose propionates would obviously be Equation 9, omitting the term $42N_a$ in the denominator, Equation 11 omitting the a term, and Equations 12 and 13 omitting the N_a terms.

Case of Cellulose Acetate Butyrate. The equations for weight per cent acetyl and butyryl are:

$$a = \frac{4300N_a}{162 + 42N_a + 70N_b} \quad (14)$$

$$b = \frac{7100N_b}{162 + 42N_a + 70N_b} \quad (15)$$

Solving Equations 14 and 15 simultaneously gives:

$$N_a = \frac{3.86a}{102.4 - 1.009b - a} \quad (16)$$

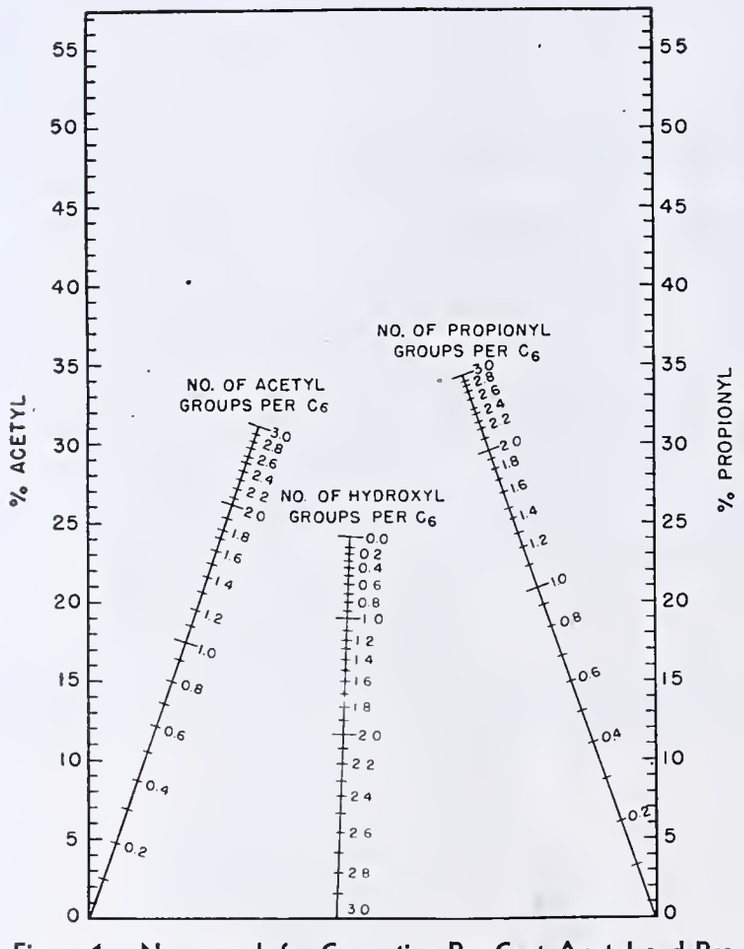

Figure 1. Nomograph for Converting Per Cent Acetyl and Per Cent Propionyl in Cellulose Acetate Propionate to Number of Acetyl and Propionyl Groups per Glucose Unit of Cellulose

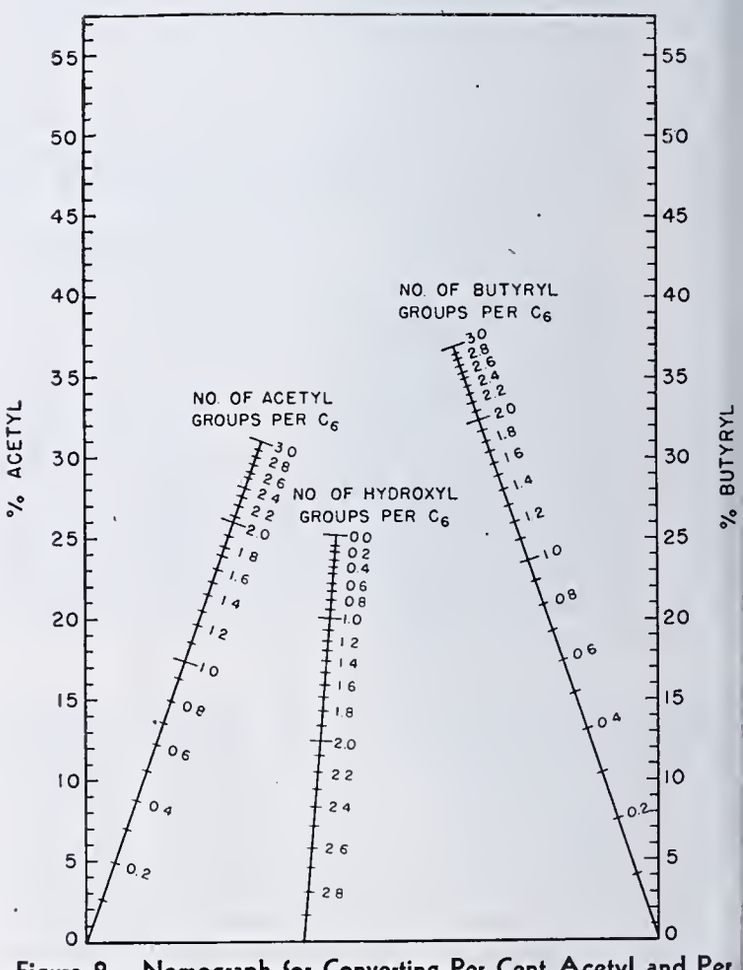

Figure 2. Nomograph for Converting Per Cent Acetyl and Per Cent Butyryl in Cellulose Acetate Butyrate to Number of Acetyl and Butyryl Groups per Glucose Unit of Cellulose

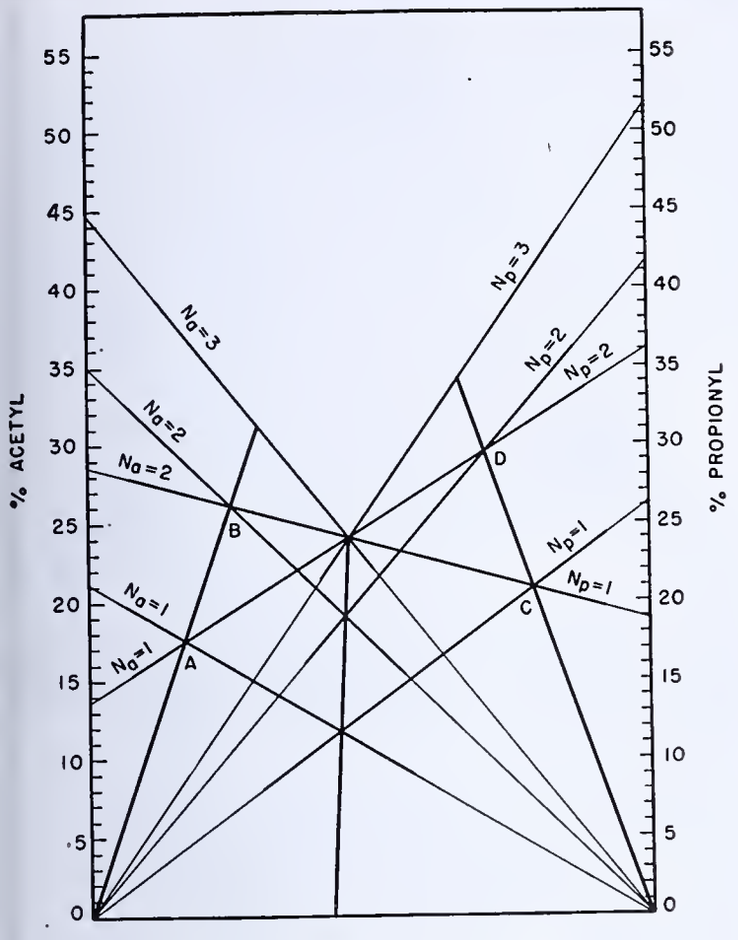

Figure 3. Construction of Nomographs

$$N_b = \frac{2.33b}{102.4 - 1.009b - a} \quad (17)$$

$$N_h = 3 - N_a - N_b \text{ by assumption} \quad (18)$$

$$h = \frac{1700N_h}{162 + 42N_a + 70N_b} \quad (19)$$

The corresponding equations for cellulose butyrates would be Equations 15, 17, 18, and 19, omitting the a and N_a terms, since both are zero in this case.

Derivation of Equations for Cellulose Acetate Propionates. The derivation of Equations 10 and 11 from Equations 8 and 9 is shown below to illustrate the method used to obtain the equations for the numbers of ester groups.

$$a = \frac{4300N_a}{162 + 42N_a + 56N_p} \quad (8)$$

$$p = \frac{5700N_p}{162 + 42N_a + 56N_p} \quad (9)$$

Equation 9 is solved for N_a and is then substituted in Equation 8 and solved to obtain the following expression for N_p:

$$N_p = \frac{162}{\frac{5700}{p} - \frac{42 \times 5700a}{4300p} - 56} \quad (20)$$

$$N_p = \frac{162}{\frac{5700}{p} - \frac{55.7a}{p} - 56} \quad (21)$$

The numerator and denominator of Equation 21 are divided by the smallest factor, 55.7, and are multiplied by p to give

$$N_p = \frac{2.91p}{102.4 - 1.006p - a} \quad (11)$$

The equation for N_a derived in the same way is

$$N_a = \frac{162}{\frac{4300}{a} - \frac{56 \times 4300p}{5700a} - 42} \quad (22)$$

Table I. Data for Construction of Nomograph

N_a	N_p	% Acetyl	% Propionyl
1	0	21.1	0
2	0	35.0	0
3	0	44.8	0
0	1	0	26.1
0	2	0	41.6
0	3	0	51.8
1	2	13.6	36.1
2	1	28.5	18.9

$$N_a = \frac{3.86a}{102.4 - 1.006p - a} \quad (10)$$

NOMOGRAPHS

Nomographs for converting per cent acetyl to the number of groups per anhydroglucose unit have been prepared (Figures 1 and 2). By this means per cent acetyl and per cent propionyl or butyryl in cellulose acetates, propionates, butyrates, acetate propionates, and acetate butyrates can be easily converted to the number of acetyl, propionyl, or butyryl, and hydroxyl groups per anhydroglucose unit.

To use these nomographs lay a ruler across the per cent acetyl value on the left scale and per cent propionyl or butyryl value on the right scale and read the number of acetyl, propionyl, or butyryl, and hydroxyl groups from the intersections with the corresponding scales of the nomograph.

Nomographs suitable for this purpose can be constructed by methods such as those of Davis (*1*) or Van Voorhis (*9*), or by the graphical method described below. Figure 3, which applies to cellulose acetates, propionates, and acetate propionates, demonstrates the method of construction used.

Lines were drawn from 0% propionyl to the points on the acetyl scale corresponding to $N_a = 1$, 2, and 3 and from 0% acetyl to the points on the propionyl scale corresponding to $N_p = 1$, 2, and 3. The data used are shown in Table I. The line showing the number of hydroxyl groups was then drawn from the intersection of the lines $N_a = 3$ and $N_p = 3$, through $N_a = 2$ and $N_p = 2$, then $N_a = 1$ and $N_p = 1$ to the base line.

The two lines located by substituting $N_a = 1$ and $N_p = 2$ and also $N_a = 2$ and $N_p = 1$ in Equations 8 and 9 were drawn next.

The line showing the number of acetyl groups was established by drawing a line from the left origin through A, the intersection

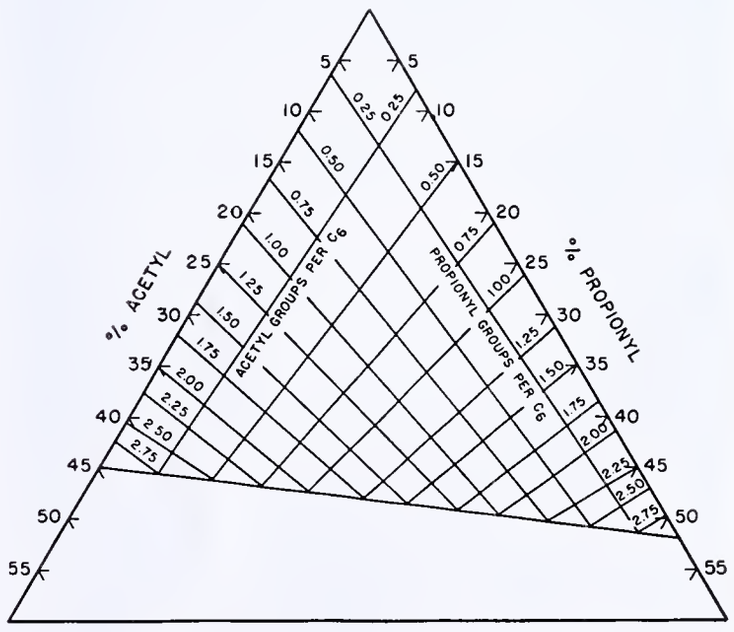

Figure 4. Relationship between Per Cent Acetyl and Per Cent Propionyl and the Numbers of Acetyl and Propionyl Groups in Cellulose Acetate Propionates

of lines $N_a = 1$ and ($N_p = 2$ and $N_s = 1$), and B, the intersection of lines $N_a = 2$ and ($N_p = 1$ and $N_s = 2$), to the line where $N_s = 3$. The line for the number of propionyl groups was drawn in the same way through points C and D.

The scales for the number of acetyl groups and hydroxyl groups were further subdivided by calculations using Equation 2 or by plotting the number of acetyl groups against per cent acetyl, and reading from this graph the acetyl values corresponding to the desired values of N_a. A straightedge was placed across these acetyl values and 0% propionyl, and the points of intersections of the acetyl and hydroxyl scales were marked. The scale for the number of propionyl groups was subdivided in the same way by using data from a corresponding graph, or by substituting values of N_p in an equation for per cent propionyl corresponding to Equation 2.

TRIANGULAR CHARTS. Malm, Fordyce, and Tanner (*4*) have correlated physical properties such as solubilities, melting points, specific gravities, and moisture absorption of these cellulose esters with their acyl contents by means of triangular diagrams. These properties can also be correlated with composition in terms of numbers of acetyl and propionyl or butyryl groups per anhydroglucose unit by means of Figures 4 and 5.

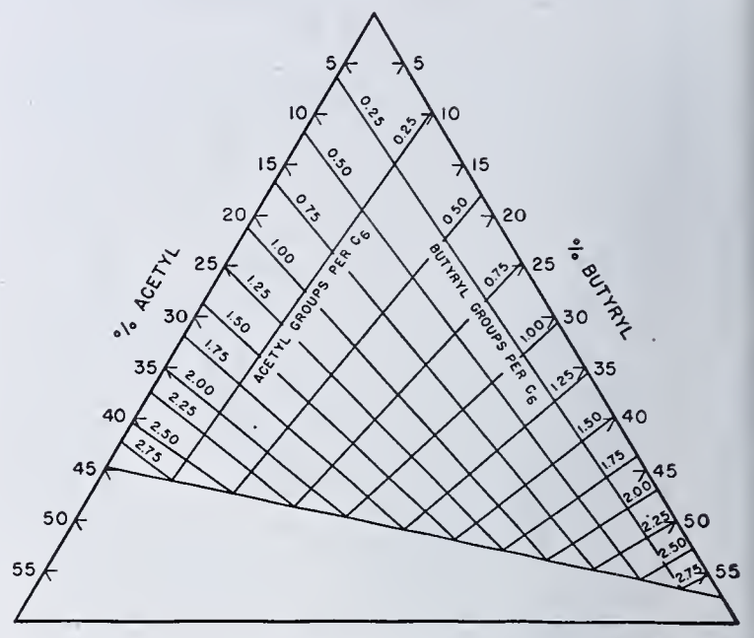

Figure 5. Relationship between Per Cent Acetyl and Per Cent Butyryl and the Numbers of Acetyl and Butyryl Groups in Cellulose Acetate Butyrates

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Determination of Cyclopentadiene and Dicyclopentadiene

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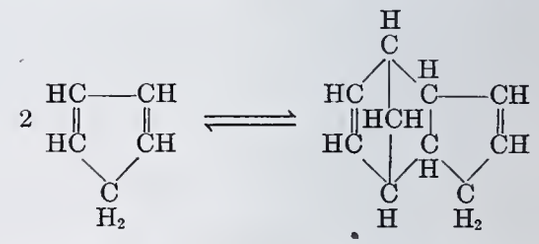
Cyclopentadiene in a C_5 fraction is determined by condensation with benzaldehyde to highly colored phenylfulvene, the intensity of the color of which is a measure of the cyclopentadiene present. None of the materials normally associated with it in hydrocarbon samples interferes. Oxidation inhibitors must be absent. Since cyclopentadiene dimerizes rapidly even at ordinary temperatures, the average C_5 fraction will contain both monomer and dimer. The latter represents available cyclopentadiene because it can easily be depolymerized, and the method described for its determination is a valuable supplement to the method for determining the monomer. The dimer is determined by controlled depolymerization to the monomer, which is determined colorimetrically as above. Cyclopentadiene originally present as such is determined separately.

AMONG the dienes which have assumed importance during recent years, cyclopentadiene is one of the most interesting. In addition to a system of conjugated double bonds, it contains an active methylene group which enables it to undergo reactions that make it of interest in the synthetic field. For a thorough review of the chemistry and utilization of cyclopentadiene, a paper by Wilson and Wells (*5*) is recommended.

One of the factors which makes cyclopentadiene such a difficult compound to work with is its instability. It can, like the other conjugated dienes, be polymerized to rubberlike materials with the aid of catalyst, but it also dimerizes spontaneously even at room temperature to form dicyclopentadiene. The rate of dimerization increases with temperature. However, if the temperature is raised to about the boiling point of dicyclopentadiene (170° C.), the reaction is reversed and the dimer reconverts to



the monomer. This means that to know the amount of cyclopentadiene originally present in the sample, it is necessary to determine both the monomer and the dimer. Methods for determining cyclopentadiene are virtually nonexistent. Those which are mentioned in the literature are not specific for cyclopentadiene and are applicable only if no other dienes are present.

A recent paper by Sefton (*2*) describes a method for determining cyclopentadiene by means of its heat of reaction with maleic anhydride. The author states that his method is not specific for cyclopentadiene, but he expects little interference, since other dienes of the same boiling range react much more slowly.

Since cyclopentadiene is a conjugated diolefin, it has a very strong ultraviolet absorption spectrum. Pickett, Poddock, and Sackter (*1*) have made a careful study of this spectrum, but have not used it for the determination of this compound. Since cyclopentadiene has a characteristic ultraviolet absorption spectrum, it could be determined rather accurately by ultraviolet spectrometry if not too many interfering materials such as aromatics and conjugated diolefins were present. Infrared spectrometry could likewise be used satisfactorily.

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A colorimetric method which is specific for cyclopentadiene has been developed in this laboratory. It is based upon the observation by Thiele (*3*) that cyclopentadiene will condense with aldehydes and ketones to form highly colored fulvenes. Ward (*4*) has utilized this reaction with acetone for the determination of cyclopentadiene, but the method is not entirely satisfactory because of the instability of the dimethylfulvene. The present method, in which benzaldehyde is used to form the fulvene, is a modification and extension of the procedure used by Ward. It can be applied to the determination of cyclopentadiene in C_5 fractions which contain no inhibitor. None of the compounds normally associated with it causes any interference.

PREPARATION OF PURE CYCLOPENTADIENE

In conducting this investigation it was necessary to have available pure cyclopentadiene. It can be conveniently prepared by depolymerizing pure dicyclopentadiene, but it cannot be stored because of its tendency to dimerize. Therefore, it must be freshly prepared each time and used immediately.

A technical grade of dicyclopentadiene (Eastman Kodak Company) was vacuum-distilled from a Claisen flask, and the fraction boiling at 82° C. at 50 mm. of mercury pressure was sufficiently pure without further treatment. As the distillate consisted largely of the endo form of dicyclopentadiene (melting point 32° C.), water at 32–33° C. was circulated through the condenser to prevent its plugging with crystals. The distilled dicyclopentadiene was stored in a brown bottle under nitrogen. It remained sufficiently pure for about a month but had to be redistilled after that period of time.

The dicyclopentadiene purified in this manner was then depolymerized by cracking in a still equipped with a 60-cm. (24-inch) tube packed with short lengths of glass rod which served as a rough fractionating column. It was provided with an air jacket to prevent excess heat loss. Before the distillation was started, the entire system was flushed with nitrogen introduced through a side arm in the flask. The flow of nitrogen was then stopped and the flask heated to the boiling point of dicyclopentadiene (about 170° C.) where depolymerization began. The rate of heating was so adjusted that the overhead vapor temperature was 40–41° C., and the distillate was caught in a receiver cooled with dry ice. A higher vapor temperature would indicate that the rate of heating was so high that dicyclopentadiene was being carried over. The heating was discontinued before the flask became dry to avoid any possible explosion from peroxides.

Occasionally the distillate was cloudy because of traces of moisture or high polymers which accumulated in the condenser, but it could be easily clarified by passing it through filter paper. This cyclopentadiene was sufficiently pure for use without any further treatment. The purity was found to be 99.1% by reaction with maleic anhydride and 100.5% by hydrogenation.

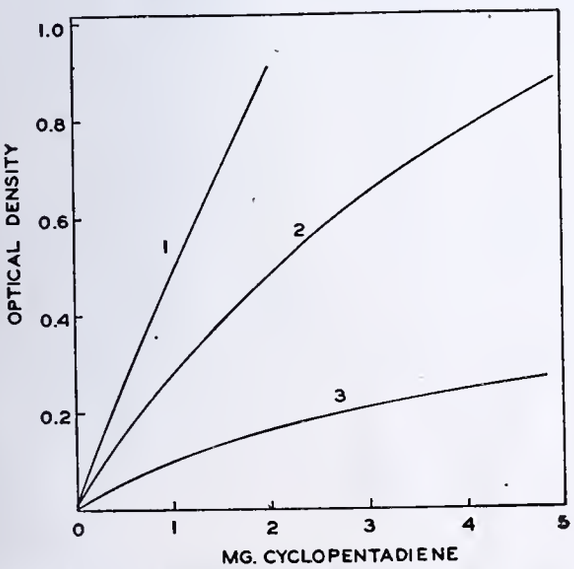

Figure 1. Calibration Curves for Various Filters

Maximum transmission, Å. 1. 4075. 2. 4250. 3. 5000

Table I. Colors and Characteristics of Fulvenes Formed from Cyclopentadiene and Various Aldehydes and Ketones

Compound	Color and Characteristics
Acetone	Yellow color becoming darker
Benzophenone	Unstable, dark red color, slow reaction
Acetophenone	Unstable, light red color
Cyclopentanone	Dark emulsion
Salicylaldehyde	Green emulsion
Anisaldehyde	Yellow color turning red
Citronellal	Yellow color becoming darker
Vanillin	Yellow precipitate
Benzaldehyde	Stable yellow to red color

Table II. Stability of Color of Phenylfulvene

Elapsed time, min.	0	15	30	60	120
Optical density	0.372	0.366	0.366	0.372	0.366

SELECTION OF REAGENT FOR DETERMINING CYCLOPENTADIENE

It is always desirable, of course, to have a method which is specific for the determination of a particular compound, and the Thiele fulvene reaction as used by Ward (*4*) appeared to be good in this respect. However, the dimethylfulvene formed by the reaction of cyclopentadiene with acetone was unstable and its color changed rapidly. Therefore, a number of other aldehydes and ketones were tested in regard to suitability of the color and stability of the fulvene. As the results in Table I indicate, phenylfulvene, the condensation product of cyclopentadiene and benzaldehyde, was the most promising. Its yellow-to-orange color is well suited to colorimetric work, and its stability, as indicated in Table II, is good. The absorption curve of phenylfulvene, as determined on a Beckman quartz spectrophotometer, shows an absorption peak of 3475 Å.

A photoelectric colorimeter, the Fisher electrophotometer, was used in developing the colorimetric method. To increase the selectivity or sensitivity, filters were used to isolate a certain region of the spectrum for measuring the optical density of the phenylfulvene solutions. Filters with maximum transmission regions below 4000 Å. could not be used with the electrophotometer because they transmitted too small an amount of energy to which the photocells were sensitive. As the wave length of the light transmitted by the filter increased, the sensitivity decreased, but there was a corresponding increase in the permissible concentration of cyclopentadiene. This is illustrated in Figure 1 by the calibration curves shown for three filters. As a compromise between sensitivity and a more extended range of concentration, the No. 2 filter was used. It will permit the determination of as much as 5 mg. of cyclopentadiene in the aliquot.

STUDIES ON THE DEPOLYMERIZATION OF DICYCLOPENTADIENE

Since cyclopentadiene is known to dimerize readily, sometimes spontaneously, the average sample, though originally a C_5 cut, will contain some dicyclopentadiene. For all practical purposes this represents available cyclopentadiene because of the ease with which it can be depolymerized to the monomer. It was apparent, therefore, that it would be necessary to have a method for determining dicyclopentadiene. Numerous chemical and physical methods were investigated for this purpose, but none was satisfactory. The depolymerization of dicyclopentadiene to cyclopentadiene was, therefore, investigated as a possible means for determining the dimer. This procedure would be satisfactory if the depolymerization could be made quantitatively, because the colorimetric method for cyclopentadiene had proved to be accurate. An investigation of the depolymerization reaction was carried out in an attempt to find conditions under which it could be made quantitative.

The depolymerization step is, of course, employed in the preparation of pure cyclopentadiene, but there no attempt is made to carry out the reaction in a quantitative manner. However, from

the experience gained in this work, it was possible to predict some of the conditions which would have to be fulfilled: (1) The depolymerization must be carried out rapidly enough so that the cyclopentadiene is removed from the heated portion of the reaction chamber before it has a chance to redimerize at the elevated temperature. (2) The rate of heating must be slow enough so that no dicyclopentadiene distills without depolymerization. (3) Some material which has a boiling point a few degrees above the depolymerization temperature of dicyclopentadiene must be used as a chaser to sweep all the cyclopentadiene completely into the cold portion of the apparatus. (4) Some high-boiling material to serve as bottoms should be used to minimize the danger of explosion if any peroxides are present.

A compromise was necessary if the first two of these conditions were to be satisfied; so various temperatures and rates and means of heating were investigated. The one which finally proved most satisfactory involved the use of an electric heater with exposed element and built-in rheostat (Precision Scientific Company 550-watt heater). The rate of heating could be controlled easily, and the depolymerization vessel could be placed directly on the heating element to obtain rapid heat transfer. Likewise, the entire vessel was heated, so that only a small amount of refluxing took place.

During the investigation to find the best conditions for depolymerization, the apparatus used in this work underwent considerable evolution. In Figure 2 is shown the form which was finally selected. The 25-ml. Erlenmeyer flask is sealed directly to the side arm and condenser, so that the cyclopentadiene vapors have only a short distance to travel before reaching the cool portion of the apparatus. The small $^{5}/_{20}$ standard-taper joint is used in rinsing out the condenser after a determination. Marks were placed at the 5- and 10-ml. points on the receiver, so that it was possible to judge more accurately the rate at which the distillate was collected.

The third and fourth conditions listed above were satisfactorily fulfilled through the use of decalin (decahydronaphthalene) as a chaser and a mineral oil of 100 Saybolt viscosity at 100° F. as bottoms. Of the materials studied, decalin was most satisfactory in regard to boiling point and stability. The exact properties of the mineral oil used as bottoms are not critical as long as its initial boiling point is well above the boiling range of the decalin used as a chaser.

DETERMINATION OF CYCLOPENTADIENE

Scope. This method is intended for C_5 fractions (18° to 50° C.) to which no oxidation inhibitor has been added. Higher boiling fractions, with the exception of dicyclopentadiene which forms from the monomer on storage, must be absent.

Apparatus. A photoelectric colorimeter, such as the AC Model Fisher electrophotometer with the appropriate cells and light filters.

An assortment of pipets, volumetric flasks, and 50-ml. Erlenmeyer flasks is required for making dilutions.

Reagents. 3% solution of alcoholic potassium hydroxide, filtered.

20% by volume solution of U.S.P. or similar grade benzaldehyde in 95% ethanol. This solution must be protected from unnecessary contact with air because the benzaldehyde is easily oxidized to benzoic acid.

Dicyclopentadiene, Eastman 80% technical grade.

Ethanol, both 95% and absolute.

Pentane, C.P.

Procedure. It is first necessary to make a standard calibration curve from pure cyclopentadiene, which is prepared from redistilled dicyclopentadiene in the manner previously described. The cyclopentadiene must be used immediately to prepare standard dilutions in C.P. pentane, and even these dilute solutions should not be used after they are more than an hour old. These solutions should be of such concentrations that they are distributed evenly throughout the range which can be covered by the particular instrument and filter used. Equal volumes of the standard cyclopentadiene solution, 3% alcoholic potassium hydroxide solution, and 20% alcoholic benzaldehyde solution are mixed and allowed to stand for 3 minutes before the optical density or per cent transmission is determined. The exact volumes of these solutions used depend entirely upon the amount of the final mixture required to fill the cell of the colorimeter. The blank or referenee cell of the colorimeter is filled with the same solutions, except that the benzaldehyde solution is omitted and an equal volume of absolute ethanol is used instead. The calibration curve is prepared by plotting the optical density or per cent transmission against the weight of cyclopentadiene for each of the standard dilutions.

The unknown samples are treated in the same manner as the known samples which were used in preparing the calibration curve. A measured amount of the sample is diluted to the proper concentration with pentane. The sample can be measured either by weighing or by using a known volume if the density is known. A blank solution should again be prepared from equal volumes of the pentane dilution of the sample, 3% alcoholic potassium hydroxide, and absolute ethanol. The reading obtained on the unknown sample is referred to the standard calibration curve to obtain the amount of cyclopentadiene present.

Since samples containing dienes usually contain peroxides, such samples should be distilled only in presence of oxidation inhibitors and of "bottoms" in the still pot. Neglect of these precautions may cause explosions. Samples containing cyclopentadiene require special care in handling, since cylopentadiene has been observed to dimerize spontaneously, sometimes explosively.

DETERMINATION OF DICYCLOPENTADIENE

Scope. This method is intended for the determination of dicyclopentadiene in the C_5 fraction. This is dicyclopentadiene which has been formed by the dimerization of some of the cyclopentadiene originally present in the sample. Other higher molecular weight compounds and oxidation inhibitors should be absent.

Apparatus. Depolymerization apparatus as shown in Figure 2. Electric heater with built-in rheostat similar to Precision Scientific Company 550-watt heater.

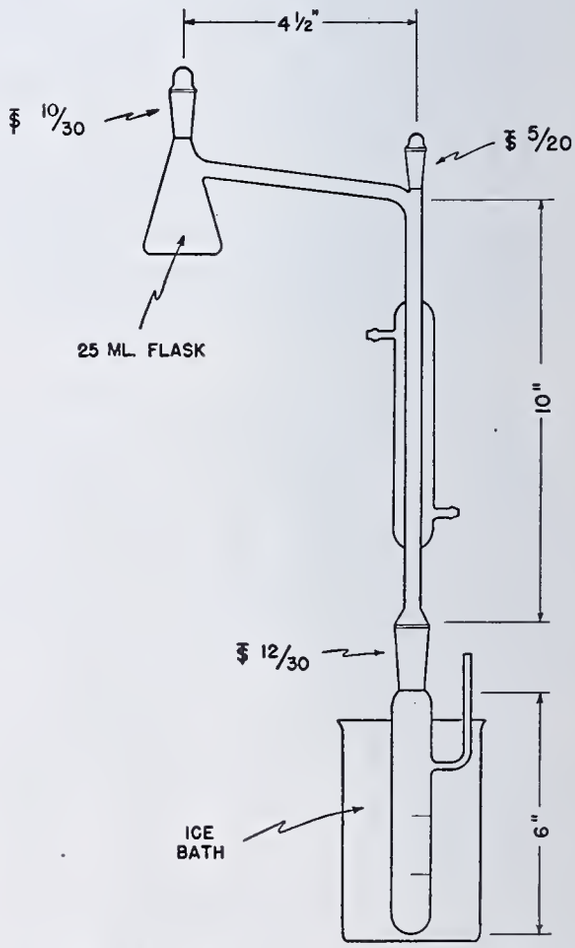

Figure 2. Apparatus for Quantitative Depolymerization of Dicyclopentadiene

Assortment of pipets and volumetric asks.
Boiling stones.

REAGENTS. Mineral oil of approximately 100 Saybolt viscosity at 100° F.
Decalin (decahydronaphthalene).
Pentane, C.P.
Ethanol, both 95% and absolute.

PROCEDURE. A 5-ml. portion of the ydrocarbon sample containing the dicyclopentadiene, 5 ml. of decalin, 5 ml. f the mineral oil, and a boiling stone re added to the 25-ml. Erlenmeyer ask of the depolymerization apparatus shown in Figure 2. If he amount of sample available is less than 5 ml. it should be rought to this volume by the addition of pentane. The electric eater is placed so that the flask rests on the heating coil, and the eceiver is surrounded by an ice bath. The heat is applied slowly, o that about 20 minutes are required to distill approximately 5 l. of C_5 hydrocarbon. The heat should then be increased so that he dicyclopentadiene is depolymerized, and decalin distills lowly. The distillation of the 5 ml. of chaser should require not ess than 40 minutes of actual distilling time (not just refluxing). When 10 ml. of distillate have been collected, the heater should e turned on high for one minute, and then the distillation topped. Some of the mineral oil will crack and an additional nilliliter or two of distillate will be collected during this time. Jnder no circumstance should the distillation be carried to dryess, because peroxides may be present.

At the conclusion of the distillation, the condenser should be insed by pouring 2 to 3 ml. of 95% ethanol through the small /20 joint, and then the entire contents of the receiver are transerred to a 100-ml. volumetric flask and made to volume with absolute ethanol. Aliquots of this solution are analyzed for cyclopentadiene by the colorimetric procedure previously described.

Table III. Colorimetric Determination of Cyclopentadiene in Pentane Solutions

Milligrams of Cyclopentadiene	
Present	Found
0.16	0.18
0.32	0.32
0.64	0.65
0.80	0.82
0.96	1.00
1.20	1.26
1.28	1.30
1.60	1.60
2.40	2.46
3.20	3.22
3.60	3.66
4.00	3.98
4.60	4.64

Table IV. Determination of Cyclopentadiene in Presence of Organic Sulfur Compounds

Cyclopentadiene		Ethyl Mercaptan Present	Thiophene Present	Methyl Sulfide Present
Found	Present			
Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*
0.8	0.8	..	..	..
2.4	2.4	..	..	..
4.0	4.0	..	..	..
0.8	0.8	2.0	..	..
2.4	2.4	6.0	..	..
4.1	4.0	10.0	..	..
0.8	0.8	..	2.6	..
2.4	2.4	..	7.8	..
4.1	4.0	..	13.0	..
1.6	1.6	..	..	17.0
3.2	3.2	..	..	34.0
1.6	1.6	1.4	1.7	5.6

Table V. Determination of Cyclopentadiene and Dicyclopentadiene in the Presence of Other C_5 Hydrocarbons

Composition of sample (% by weight)					
Pentane	73.2	73.8	47.3	23.9	66.5
Pentene-2	9.9	9.9	19.7	18.9	9.8
Piperylene	4.2	6.3	10.3	19.9	4.7
Isoprene	3.9	1.9	9.9	19.6	5.1
Cyclopentadiene	2.5	4.9	7.3	11.8	6.1
Dicyclopentadiene	6.3	3.2	5.4	5.8	7.8
Found (% by weight)					
Cyclopentadiene	2.5	4.9	7.6	11.8	5.9
Dicyclopentadiene	6.2	3.2	5.2	5.6	7.7

Table VI. Change in Cyclopentadiene Content of Sample on Storage

Composition of original sample (% by weight)
18.8% cyclopentadiene
14.2% dicyclopentadiene
67.0% C_5 fraction of fluid catalytically cracked naphtha

Weight % found after days	0	2	3	4	5	6	7	9	12	16	30
Cyclopentadiene	18.8	16.1	14.3	12.5	11.5	10.6	9.6	8.0	6.5	5.6	2.9
Dicyclopentadiene	14.2	17.4	18.7	20.5	21.5	22.4	23.4	25.0	26.5	27.4	30.1
Total (as cyclopentadiene)	33.0	33.5	33.0	33.0	33.0	33.0	33.0	33.0	33.0	33.0	33.0

Dicyclopentadiene is determined as the difference between the total cyclopentadiene thus determined and the cyclopentadiene present as such in the original sample.

ACCURACY OF METHODS. The colorimetric method for determining cyclopentadiene appears to be capable of a rather high degree of accuracy. The results of a series of analyses on samples made from pentane and cyclopentadiene are shown in Table III. Here the maximum error is 0.06 mg. of cyclopentadiene, and the average error is 0.03 mg. or 0.3% on the basis of the original sample. As far as has been determined at the present time, the method is specific for cyclopentadiene, and certainly none of the materials which would normally be associated with it in a hydrocarbon sample causes any interference. Some of the sulfur compounds, ethyl mercaptan, methyl sulfide, and thiophene, were tried and found to be without effect (Table IV).

The only additional step involved in the determination of dicyclopentadiene is, of course, the depolymerization reaction. This reaction can be carried out quantitatively if sufficient care is exercised, and the dicyclopentadiene can be determined accurately. In Table V are given the results of analyses made on samples containing both the monomer and dimer blended with other C_5 hydrocarbons. Isoprene and piperylene were present in each of these samples, and, since the results were still accurate, it is apparent that these other conjugated C_5 dienes do not interfere.

Additional proof of the accuracy and precision of the method, as well as an indication of the need for the method for dicyclopentadiene, is shown in Table VI. The sample was prepared by adding a known amount of cyclopentadiene and dicyclopentadiene to the C_5 fraction of a fluid catalytically cracked naphtha, which was found by analysis to be free of cyclopentadiene. This sample was stored at room temperature in a dark bottle and portions of it were analyzed after various periods of time. After only 2 days a very appreciable change had occurred, and after 30 days practically all of the cyclopentadiene had dimerized. It will be noticed that in each of these analyses, with the exception of the one on the second day, the total of the monomer and dimer was 33.0% or exactly the theoretical amount. This rigorous test of the accuracy and reproducibility of the methods indicates that they are entirely reliable.

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Rapid Photometric Determination of Iron and Copper in Red Phosphorus

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Photometric methods for the determination of iron and copper as colored complexes with 1,10-phenanthroline and diethyldithiocarbamate, respectively, can be adapted to the rapid determination of traces of iron and copper in red phosphorus with a precision of 2 p.p.m. for either component in the range 0 to 50 p.p.m. Interference from aluminum is minimized by addition of ammonium citrate. For solution of red phosphorus, oxidation with a bromine–carbon tetrachloride mixture is recommended in the analysis for iron, whereas oxidation with nitric acid is recommended in the analysis for copper.

THE stability of red phosphorus toward atmospheric oxidation is decreased markedly by traces of iron and copper. The critical stability requirement involved in certain uses of red phosphorus reflects a need for precise, and preferably rapid, methods for the determination of contaminative iron and copper. Several methods for the determination of traces of iron and copper have been published, but none is specifically applicable to the analysis of red phosphorus. Yoe and Sarver (*6*) have prepared a bibliography of organic reagents for the determination of iron and copper. From a comparison of several photometric methods for iron, Woods and Mellon (*5*) have recommended the 1,10-phenanthroline method (*2*) for general application. Haywood and Wood (*3*) have used the color of the cupric diethyldithiocarbamate complex as a basis for the determination of copper in ferrous materials that had first been dissolved in a mixture of phosphoric and sulfuric acids.

In the present work, conditions were established for dissolving red phosphorus preparatory to its photometric analysis for iron as the ferrous 1,10-phenanthroline complex and for copper as the cupric diethyldithiocarbamate complex. The methods of Fortune and Mellon (*2*) and of Haywood and Wood (*3*) were adapted to the formation of the respective colored complexes. The influence of interfering ions and of other factors on the development of color was studied.

Red phosphorus dissolves in dilute nitric acid to form a solution of mixed acids of phosphorus. On evaporation of the solution to small volume and addition of concentrated nitric acid, the lower acids of phosphorus are oxidized to a mixture of ortho-, meta-, and pyrophosphoric acids. Pyro- and metaphosphoric acids do not affect the determination of copper but do affect development of color in the determination of iron. Although both of these acids can be hydrolyzed to orthophosphoric acid, the iron extracted from the glass vessel during the oxidation step is additive in the iron analysis. Nitric acid thus is a suitable solvent for the determination of copper but not for the determination of iron.

A solution of bromine in carbon tetrachloride reacts smoothly with red phosphorus to form crystals of an addition compound of phosphorus pentabromide and carbon tetrachloride (*4*). Upon the addition of water and subsequent evaporation of the carbon tetrachloride, the phosphorus pentabromide hydrolyzes to orthophosphoric and hydrobromic acids. The hydrobromic acid is decomposed by nitric acid. This method for dissolution of the sample obviates the disadvantages involved in the use of nitric acid as the solvent in an iron determination.

Many samples of commercial red phosphorus yield a yellow to brown solution. The objectionable color is bleached by the action of potassium chlorate on the hot sirupy solution of phosphoric acid.

In the analysis of red phosphorus that has been coated with hydrous alumina (aluminated) to increase its stability, the aluminum precipitates as the phosphate and interferes with the determination of both iron and copper. Ammonium citrate prevents precipitation of the aluminum and can be used without special precautions in the copper determination. In the iron determination, however, tests similar to those of Cowling and Benne (*1*) confirmed their observation that the citrate ion markedly retards the formation of the ferrous 1,10-phenanthroline complex. In tests with 30 mg. of aluminum hydroxide per gram of phosphorus, a 1 to 1 molar ratio of citric acid to aluminum hydroxide proved effective. A slightly greater amount of citric acid is specified in the procedure to take care of normal variations in samples of red phosphorus. Increase of the additions of reducing and color-forming reagents over the amounts used by Fortune and Mellon (*2*) eliminates most of the interference by the citrate.

APPARATUS AND REAGENTS

Filter photometer, Fisher AC electrophotometer fitted with 425-mμ blue and 525-mμ green filters and with 2- and 5-cm. absorption cells.

Nitric acid, 70%, redistilled.

Bromine–carbon tetrachloride mixture, 1 volume of bromine to 3 volumes of redistilled carbon tetrachloride.

Citric acid, 10 grams of the monohydrate per 100 ml. of solution.

Hydroxylamine hydrochloride, 10 grams per 100 ml. of solution.

1,10-Phenanthroline, 0.1 gram per 100 ml. of solution.

Colloid, 1.0 gram of gum acacia dissolved in 100 ml. of boiling water, filtered, and diluted to 200 ml. Prepare fresh daily.

Sodium diethyldithiocarbamate, 0.2 gram per 100 ml. of solution, stored in a dark bottle.

Standard iron solution, 1 ml. ≈ 0.01 mg. of iron. Dilute to 1 liter a 100-ml. aliquot of a solution containing 0.7021 gram of ferrous ammonium sulfate hexahydrate and 5 ml. of sulfuric acid per liter.

Standard copper solutions. Dissolve 0.2500 gram of pure copper in nitric acid and dilute to 1 liter. Dilute 100 ml. of this stock solution to 1 liter for standard solution A (1 ml. ≈ 0.025 mg. of copper). Dilute 200 ml. of solution A to 1 liter for standard solution B (1 ml. ≈ 0.005 mg. of copper).

Potassium chlorate, reagent grade.

Indicator. Alkacid test paper (manufactured by Fisher Scientific Company).

DETERMINATION OF IRON

PREPARATION OF CALIBRATION CURVES. To a series of 100-ml. volumetric flasks add 0 to 28 ml. of the standard iron solution in 2-ml. increments. To a second series of the flasks add, from a microburet, 0 to 5.00 ml. of the standard iron solution in 0.20-ml. increments. Into the contents of each flask mix 1 ml. of hydroxylamine hydrochloride solution, and allow the mixture to stand for 15 minutes. Add 5 ml. of 1,10-phenanthroline solution, and adjust the pH to 6 by dropwise addition of dilute ammonium hydroxide. This is best accomplished by touching a drop of the solution on a stirring rod, to a piece of Alkacid test paper. Dilute each solution to the mark, mix it thoroughly, and allow 1 hour for color development. Read the color intensity on a photometer equipped with a 525-mμ (green) filter. For the solutions in the first series, which represent 0 to 280 p.p.m. of iron on the basis of a 1-gram sample of red phosphorus, use 2-cm. cylindrical cells; for those in the second series, which represent 0 to 50 p.p.m. of iron, use 5-cm. rectangular cells. Plot color intensity versus p.p.m. of iron.

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Blank Determinations. Determine the iron content of each w preparation of bromine–carbon tetrachloride mixture. ilute 1 volume of phosphoric acid of known iron content with 19 lumes of distilled water, and measure 44.0 ml. of the solution m a buret into each of six 250-ml. beakers. Add the same antities of bromine–carbon tetrachloride mixture and other agents that are used in a regular analysis of red phosphorus, d put the blanks through the same analytical procedure. Subact the iron content of the phosphoric acid from the total iron und to obtain the blank on the bromine–carbon tetrachloride ixture. Subtract the average blank thus obtained from the tal iron found in the analysis of red phosphorus to obtain the ue iron content of the red phosphorus.

When a large excess of hydroxylamine hydrochloride is used in e determination of iron in aluminated red phosphorus, run a parate blank on this reagent. To 10 ml. of the hydroxylamine ydrochloride solution add 5 ml. of 1,10-phenanthroline solution d adjust the pH to 6 by dropwise addition of dilute ammonium ydroxide. Dilute the solution to 100 ml., mix thoroughly, and low 1 hour for color development. Use three-tenths of the iron und as a correction in the analysis of aluminated red phosnorus.

Procedure. Weigh 1.00 gram of red phosphorus into a dry 50-ml. beaker. Successively add 75 ml. of carbon tetrachloride, 5 ml. of distilled water, and 15 ml. of nitric acid. Place the mixre under a hood, and add 20 ml. of bromine–carbon tetraloride solution, from either a buret or a pipet equipped with a ction bulb, at a rate that will not cause violent reaction. Stir e mixture until formation of yellow crystals of $PBr_5.2CCl_4$ ases. Then nest the 250-ml. beaker and its contents in a 400-l. beaker with the lips of the beakers divergent. In the air ath thus formed the mixture can be evaporated with fair rapidity d without bumping.

Evaporate the liquid on a hot plate at medium temperature unl frothing and evolution of dense brown fume occur at a volume 5 or 6 ml. If the liquid is colored, add a few potassium lorate crystals and continue the heating until decolorization sues. Avoid prolonged heating lest the beaker be attacked.

Dilute the solution to 100 ml. with distilled water, cover with a yrex watch glass, and boil at a rate that will decrease the volume about 30 ml. in 45 minutes. Transfer the solution to a 100-ml. olumetric flask, rinse the beaker five or six times with 5-ml. ortions of distilled water, and add the washings to the flask. Dilute the solution to 70 ml. The remainder of the procedure epends upon whether the original sample of red phosphorus was luminated.

If the original sample was unaluminated, add 1 ml. of hydroxylmine hydrochloride solution to the flask. After 15 minutes add ml. of 1,10-phenanthroline solution, and adjust the pH to 6 y dropwise addition of ammonium hydroxide. Dilute the solution to the mark, mix it thoroughly, and allow the color to develop or 1 hour. Read the color intensity on a photometer with a 525-mμ (green) filter. If color intensity indicates more than 50 .p.m. of iron, use 2-cm. cylindrical cells; if less than 50 p.p.m., se 5-cm. rectangular cells. Read the iron content from the ppropriate calibration curve. Correct for the blank on the eagents.

If the original sample was aluminated, add 4 ml. of hydroxylmine hydrochloride solution to the solution of dilute orthohosphoric acid in the 100-ml. volumetric flask. After 15 inutes add 1.0 ml. of citric acid solution, shake the mixture thorughly, and cool it to room temperature. Add 10 ml. of 1,10-henanthroline solution, and adjust the pH to 6 by dropwise ddition of ammonium hydroxide. Dilute the solution to the nark, mix it thoroughly, and allow 2 hours for color to develop. Read the color intensity and determine the iron content as described for unaluminated red phosphorus.

Factors Affecting Color Development. The presence of rthophosphoric acid narrows the pH range for maximum color levelopment. At the optimum pH of 6 ± 1, however, quantities f orthophosphoric acid equivalent to as much as 2 grams of red hosphorus are without effect.

The conditions specified in the procedure for aluminated red hosphorus practically overcome interference from the citrate hat is added to prevent precipitation of aluminum.

Lead, which may be present in red phosphorus, forms a precipitate. In aluminated samples to which the specified quantity f citrate has been added, up to 1500 p.p.m. of lead can be tolerated.

Neither nickel nor copper interferes in proportions less than 100 p.p.m. Larger proportions of copper cause high results.

Precision and Accuracy. The precision of the method was tested by analyzing in multiple a sample of pure red phosphorus, both alone and with known additions of iron. From 32 determinations in which the maximum deviation from the mean was 3 p.p.m., the standard deviation of a single value was shown to be 1.2 p.p.m. of iron.

To test the precision of the method on aluminated samples, 12 samples containing the equivalent of 30 mg. of aluminum hydroxide as the nitrate were analyzed. The maximum deviation from the mean was 2.7 p.p.m., and the standard deviation was 1.6 p.p.m. of iron. The accuracy is believed to be as good as the precision. Occasional discrepancies in the analysis of less pure samples were shown to be due to inhomogeneity of the samples.

DETERMINATION OF COPPER

Preparation of Calibration Curves. To a series of 100-ml. volumetric flasks add, in increments of 1 ml. from a microburet, from 0 to 12 ml. of standard copper solution A (1 ml. ≈ 0.025 mg. of copper) and from 0 to 10 ml. of standard copper solution B (1 ml. ≈ 0.005 mg. of copper). Then add to each flask 10 ml. of 3 *N* ammonium hydroxide and 10 ml. of the suspension of gum acacia. Dilute the mixture to about 70 ml., agitate it thoroughly, add 10 ml. of sodium diethyldithiocarbamate solution, and make the volume up to 100 ml. Allow 15 minutes for color development. Read the color intensity on a photometer fitted with a 425-mμ (blue) filter. For the solutions prepared from standard copper solution A, which represent 0 to 120 p.p.m. of copper on the basis of a 2.5-gram sample of red phosphorus, use 2-cm. cylindrical cells; for those prepared from standard copper solution B, which represent 0 to 20 p.p.m. of copper, use 5-cm. rectangular cells. Plot color intensity versus p.p.m. of copper.

Procedure. Weigh a 2.50-gram sample of red phosphorus into a 300-ml. tall-form beaker, and add 100 ml. of nitric acid (1 + 1). Heat the mixture gently to start the reaction, then allow the reaction to proceed in the cold. After the vigorous reaction subsides, evaporate the liquid until the evolution of brown fume ceases. Add 2 to 5 ml. of concentrated nitric acid to the cooled solution and reheat it until the evolution of brown fume ceases. Repeat the cycles of adding nitric acid and heating until a cycle fails to produce brown fume, then evaporate the liquid to a sirup. If the sirup is colored, heat it for a few minutes with about 0.2 gram of potassium chlorate. Add 10 to 20 ml. of water to the cooled solution and reheat it to boiling to remove chlorine and oxides of nitrogen. If the solution is not colorless after boiling for 10 minutes, re-evaporate it to a sirup and repeat the potasium chlorate treatment.

Transfer the degassed solution to a 100-ml. volumetric flask, and add 2.0 ml. of citric acid solution. Add ammonia until a drop of the solution turns Alkacid test paper a distinct green (pH > 8.5). Thoroughly mix 10 ml. of the suspension of gum acacia with the ammoniacal solution, and dilute the mixture to 70 ml. Add 10 ml. of sodium diethyldithiocarbamate and sufficient water to make 100 ml. Measure the color intensity with the photometer, using 5-cm. rectangular cells for 0 to 20 p.p.m. of copper and 2-cm. cylindrical cells for 20 to 120 p.p.m. of copper. Determine the p.p.m. of copper from the appropriate calibration curve.

Factors Affecting Color Development. The pH must be at least 8.5 for proper color development. A moderate excess of ammonium hydroxide does not interfere with color development, but a large excess precipitates diammonium phosphate. Phosphorus is without effect. The added citric acid eliminates interference from aluminum and decolorizes traces of iron which might otherwise cause a slight positive error.

Significant errors can be introduced by a few elements that form precipitates or colored complexes. Nickel forms a greenish-yellow complex with sodium diethyldithiocarbamate. The removal of nickel with dimethylglyoxime, as recommended by Haywood and Wood (*3*), introduces an error of about 0.015 mg. of copper (6 p.p.m. on basis of a 2.5-gram sample), probably because of the slight solubility of nickel dimethylglyoxime. It is recommended that nickel, if present, be removed with dimethylglyoxime and that a correction be made for the positive error introduced. Lead in amounts greater than 40 p.p.m. in the red phosphorus interferes by precipitation. None of the other known interfering elements has been detected in red phosphorus.

PRECISION AND ACCURACY. Since red phosphorus of known copper content was not available for analysis, the accuracy of the method could not be determined. The precision of the method was 2 p.p.m. of copper on a sample that analyzed 33 p.p.m. The precision was unaltered by the addition of sufficient standard copper solution to raise the copper content of the sample to 80 p.p.m. Results reproducible within 0.2 p.p.m. have been obtained on samples of red phosphorus that contained less than 10 p.p.m. of copper.

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Simplified Determination of Manganese in Caustic Soda

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A simple and rapid method for the determination of the traces of manganese in commercial caustic soda is described. By the use of visual comparison instead of photoelectric colorimetry the method is made more flexible for use in industrial control laboratories. The precision expressed as LU_2 was ±0.11 p.p.m. on a sample containing 0.30 p.p.m. of manganese.

IN A recent article (*2*) Williams and Andes described two methods for determining the small concentrations of manganese usually present in commercial caustic soda. The direct method developed the permanganate color by oxidizing with periodate the manganese in 20 grams of 50% sodium hydroxide, the color being measured in an Eimer and Amend photoelectric colorimeter on the 100 ml. of solution finally obtained. The extraction method involved the extraction of the manganese salt from 100 grams of 50% sodium hydroxide by a chloroform solution of 8-hydroxyquinoline, the reagent subsequently being destroyed and the manganese oxidized, after which the permanganate color was measured by the colorimeter also in 100 ml. of solution. The extraction method had an improved precision about in the ratio that would be expected from the use of a fivefold increase in sample size. Unfortunately this increase in precision, while necesssary for some exact analyses, was obtained at the expense of increased complexity and required time for the analyses. The direct procedure had an LU_1 (*1*) of ±0.18 p.p.m. which could be obtained only through very careful use of a sensitive photoelectric colorimeter because the developed pink colors were so weak.

It was necessary to devise a rapid and simple method for manganese which could be applied in laboratories that did not possess a sensitive colorimeter. Although the colors developed by the direct method were weak, they were visible to the eye, which led to the attempt to adapt the method to Nessler-tube comparison. After some experimentation such a method was developed.

SPECIAL APPARATUS AND REAGENTS

Phosphoric acid, 1 to 1. Equal volumes of C.P. 85% orthophosphoric acid and distilled water.

Treated phosphoric acid, 1 to 10. To 1 liter of distilled water add 100 ml. of 85% phosphoric acid. Place in a 2-liter beaker, add 0.8 gram of potassium periodate, and boil vigorously for 20 to 25 minutes. This boiling should reduce the volume to about 1 liter. Cool and preserve in a very clean glass-stoppered bottle which has been rinsed with the reagent. Do not add water after boiling.

Standard manganese solution. Dissolve 0.3077 gram of manganous sulfate monohydrate in water, add 10 ml. of 85% phosphoric acid, and dilute to 1 liter in a volumetric flask. Dilute 10 ml. of this solution to 1 liter. One milliliter of this second solutic contains 1 microgram of manganese.

Nessler tubes. A set of matched 12-inch 100-ml. Nessler tub should be used. These tubes should have optical glass flat bo toms and be held in a stand having an opaque white base.

PROCEDURE

Weigh 20 ± 1 grams of 50% sodium hydroxide (or the equiv lent amount of other concentrations) into a 250-ml. wid mouthed Erlenmeyer flask and dilute with 50 ml. of water. Ac 50 ml. of 1 to 1 phosphoric acid and 0.8 gram of potassium peri date. Heat the solution to boiling and boil vigorously for 20 to minutes, so that 50 to 70 ml. remain at the end of the boili period. Cool to room temperature and dilute to 100 ml. wi treated phosphoric acid in a 100-ml. graduated cylinder. F comparison with knowns, transfer to a Nessler tube that has be rinsed with about 10 ml. of the treated phosphoric acid.

Simultaneously with the samples, prepare a set of standar by adding 4, 6, 8, and 10 ml. of the 1 microgram per ml. standa manganese solution, equivalent to 0.2, 0.3, 0.4, and 0.5 p.p.r of manganese, to a series of 250-ml. wide-mouthed Erlenmey flasks. Proceed as above, beginning with the dilution with ml. of water. Compare colors and report samples to the neare 0.05 p.p.m. These standards should cover the range of mo samples of commercial liquid caustic soda but, if the samp color is lighter or darker, prepare additional standards.

DISCUSSION

It was found essential to use the treated phosphoric acid for dilutions to avoid fading. The very small concentrations of pe manganate present in the final solution, though easily perceptib to the eye, are very sensitive to destruction by slight traces reducing matter. If the treated acid was boiled vigorously a all apparatus kept clean, no difficulty was encountered with fa ing. A few time studies indicated that it was preferable to u the standards for one day only, however.

This method was adaptable to the analysis of large groups samples, the number being limited only by the size of the h plate that was used. It was found simple enough to be plac in the hands of laboratory technicians having but minimu amounts of training. The use of a reagent blank was not nece sary because standards and samples were prepared with the san reagents.

ACCURACY AND PRECISION

The method was tested for precision under routine conditio (*1*) by allowing seven routine analysts, previously unacquaint with the method, to make a total of 21 determinations on o sample of commercial 50% caustic soda. An average of 0. p.p.m. was obtained with an LU_2 of ±0.11 p.p.m. No sing result differed from the average by more than 0.05 p.p.m. To te the accuracy, the same sample was carefully analyzed by t

direct method (*2*) to obtain values of 0.30 and 0.33 p.p.m., and by the extraction method (*2*) to obtain results of 0.31 and 0.33 p.p.m. All results agreed with the average within the precision of the methods.

All precision criteria mentioned in this article were obtained by the techniques described by Moran (*1*). The LU_1 results, or limits of uncertainty under ideal conditions, were calculated from the results of ten replicate analyses made by one experienced chemist at one time on one sample, the LU_1 being expressed as three standard deviations. The LU_2 result, or limit of uncertainty under routine conditions, represents three standard deviations derived from a larger number of analyses made by routine technicians at various times on the same sample. Obviously the LU_2 test is open to more variations than the LU_1 test. When the LU_2 result on the currently described method was found satisfactory, no necessity for obtaining an LU_1 was seen.

A series of 17 consecutive tank-car shipments of 50% caustic soda was analyzed by both the authors' laboratory and the laboratory of the consignee of the shipments. The averages of the two series of tests were 0.33 and 0.32 p.p.m., with no result on any one shipment differing by more than 0.05 p.p.m.

It may seem somewhat surprising that by substituting visual comparison for instrument comparison an actual gain in precision was obtained. The limiting precision of the direct method (*2*) under the best conditions was ±0.18 p.p.m. while the present method had a precision of ±0.11 p.p.m. under routine conditions. While the modifications introduced into the current method may have improved precision somewhat, it is the authors' belief that the normal human eye may be superior for detecting slight changes in very light colors unless an exceptionally stable and sensitive photoelectric colorimeter is available.

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Determination of Total Beta-Carotene in Sweet Potatoes and Sweet Potato Products

An Improved Method

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A new method for the routine determination of carotene in sweet potatoes, sweet potato products, and by-products has been evolved from study of the various previously proposed methods. Consideration of the three essential steps of carotene analysis—i.e., extraction, purification, and spectrophotometric measurement—led to the selection of ethyl alcohol as a solvent for the extraction; of a dicalcium phosphate adsorption column for the purification; and of a synthetic hydrocarbon as a solvent for use in the spectrophotometric measurements.

DURING the course of analysis of a large number of samples incident to investigations on the methods of commercial extraction of carotene from sweet potatoes (*24*) and by-products of sweet potato starch manufacture (*1*), and on the stability of carotene in dehydrated sweet potatoes during storage (*2*), it became evident that no completely satisfactory method for the routine determination of carotene in sweet potatoes and sweet potato products and by-products is available in the literature. Consequently, detailed examinations have been made of the three essential steps in the procedure: (1) extraction of the carotene, (2) purification of the extracted carotene, and (3) spectrophotometric measurement of the finally purified carotene.

This communication presents the procedure finally adopted for the routine determination of total β-carotene in sweet potatoes and sweet potato products, evolved from studies and tests made of the various proposed changes in different steps in the procedure which have appeared in the literature. Though the procedure is especially adapted to sweet potatoes and sweet potato products, the section dealing with spectrophotometric measurement should be satisfactory for the evaluation of total β-carotene extracted and purified from other natural products. It is recognized that a variety of extraction and purification procedures are required for satisfactorily analyzing different products for β-carotene. However, the basic observations outlined regarding extraction and purification are equally applicable to the analysis of any material for carotene.

ANALYTICAL PROCEDURE

Weigh into 250-ml. ground-glass-stoppered Erlenmeyer flasks 0.5 to 5.0 grams of the finely divided sweet potato or sweet potato product. A food chopper provides a convenient method for grinding the sample. A portion of the ground sample from the chopper should be retained for moisture determination. The sample size will, of course, depend upon the expected carotene content. The final solution should contain approximately 2 mg. of carotene per liter for measurement through a 1-cm. cell. For fresh sweet potatoes a 5-gram sample, for dehydrated sweet potatoes a 2.5-gram sample, and for the high carotene-containing coagulum described elsewhere (*1*) a 0.5-gram sample will be satisfactory. If the sample is dehydrated, reconstitution by boiling in the Erlenmeyer flask for 30 minutes with about 40 ml. of water should precede the extraction.

Add 100 ml. of cold 95% ethyl alcohol to the Erlenmeyer flask and extract the carotene by shaking with a mechanical shaker for 30 minutes. Filter the extract through a sintered-glass funnel, using vacuum. Use 90% methyl alcohol followed by a small quantity of iso-octane to effect the transfer from the flask to the funnel. Wash the solid material on the sintered-glass funnel with small portions of the same two solvents. Transfer the filtrate quantitatively to a separatory funnel.

Grind in a mortar to a fine powder the solid material remaining on the sintered-glass funnel and re-extract as before with ethyl alcohol. Continue the extraction until a colorless extract is obtained and the residue is snow-white. Usually, a second extraction is sufficient for a fresh sweet potato. For a dehydrated sample a third extraction may be required, while a very high-carotene coagulum may require additional extractions.

Add 100 ml. of water and 2 to 3 grams of sodium chloride to the separatory funnel containing the combined extracts. Shake, and when the two phases have separated, transfer the alcohol-water layer to a second separatory funnel. Extract this solution with

Table I. Comparison of Alcoholic Potassium Hydroxide Refluxing and Cold Alcohol Shaking Methods for Extraction of Carotene

Sample No.	Carotene Content, Alcoholic Potassium Hydroxide Refluxing *Mg./kg.*	Carotene Content, Cold Alcohol Shaking *Mg./kg.*
1	110.0	125.0
2	116.5	127.5
3	32.8	36.0
4	142.1	164.7
5	159.0	198.0
6	91.5	115.0
7	98.8	112.5

a 25- to 30-ml. portion of iso-octane, adding the iso-octane layer to the first separatory funnel. Continue this partition with fresh portions of iso-octane until the iso-octane remains colorless, combining these extracts in the first separatory funnel.

Filter the iso-octane solution through anhydrous sodium sulfate and then pour the entire solution, or an aliquot thereof, through an adsorption column of dicalcium phosphate and Dyno, previously prepared by the methods described by Moore (*14*). Collect the filtrate from the column in a volumetric flask. Wash the column with iso-octane and dilute the filtrate to a volume containing approximately 2 mg. per liter for measurement through 1-cm. cells. Use correspondingly more dilute solutions if longer cells are used. Measure the optical density of this solution in a spectrophotometer at 450 and 476 mμ. Calculate the carotene content of the sample from the equations:

$$\text{Carotene, mg. per kg.} = \frac{\text{optical density at 450 m}\mu}{245} \times \frac{1{,}000{,}000}{cl}$$

$$\text{Carotene, mg. per kg.} = \frac{\text{optical density at 476 m}\mu}{219} \times \frac{1{,}000{,}000}{cl}$$

where c is the concentration of the sample in the final aliquot measured, expressed in grams per liter, and l is the length, in centimeters, of the absorption cell used for the measurements. Report the average of the two calculations, which should not vary by more than 0.2 to 0.3%, as the carotene content of the sample.

EXTRACTION OF CAROTENE

A complete extraction of the unchanged carotene must be effected for a satisfactory determination of carotene in the sweet potato. The hot alcoholic potash extraction procedure of Peterson, Hughes, and Freeman (*18*), based on the earlier work of Willstätter and Stoll (*26*) and of Guilbert (*8*), has probably been the basis for the most commonly used procedure. It has been shown by Lease and Mitchell (*11*) and confirmed by Fraps, Meinke, and Kemmerer (*5*) that in the saponification step the alcoholic potash reacts with the sweet potato substance, yielding a resinous material from which the carotene can be extracted only with difficulty, and then never completely. It is well known that carotene is isomerized by alkalies. Carter and Gillam (*3*) and Zechmeister and Tuzson (*29*) have shown clearly that heating solutions containing carotenes will also cause considerable isomerization. It is, therefore, essential for a satisfactory determination of carotene in the sweet potato that a complete extraction of the unchanged carotene be carried out without saponification and without heating. Of various solvents tested for their relative efficiency in the extraction of carotene in the cold, ethyl alcohol (95%) proved the most satisfactory. This is the solvent used by Lease and Mitchell (*11*) and recently recommended by the Association of Official Agricultural Chemists in proposed changes to make their method for crude carotene in dried hays applicable to sweet potatoes (*10*). To permit simultaneous extraction of from 6 to 12 samples, a mechanical shaker was preferable to Waring Blendors. In the procedure recommended the solution from the last extraction should be colorless and the residues should be snow-white. (In the extraction of carotene from sweet potato material by saponification methods a white residue cannot be obtained.) Any chlorophyll retained in the solution will be removed in the purification step. Values for carotene obtained from the same samples by the saponification and by the cold alcohol extractions are compared in Table I. The purification and method of final evaluation were identical.

PURIFICATION OF EXTRACTED CAROTENE

The two general methods by which the iso-octane solution may be purified by removal of other carotenes, carotenoid pigments, and chlorophyll are phasic and chromatographic separations. Independent experiments described by many workers have shown clearly that phasic separations are not quantitative (*4*, *13*). The use of chromatographic techniques has been studied by many and summarized by Zechmeister (*27*) and by Zechmeister and Cholnoky (*28*). Complete chromatographic analysis involving the separation of the various isomers is not practical for routine analytical procedures (*16*). It is preferable that the adsorbent retain noncarotene impurities and permit the carotene in solution to pass through the column, completely purified and ready for final spectrophotometric measurement. This requirement is met in the so-called liquid chromatographic column technique of Zechmeister (*27*). Study of many adsorbents led to the selection of dicalcium phosphate, recommended by Moore (*13*). For reasons given below, petroleum ether solvents or the so-called "pure" hexanes and heptanes obtained from them, were considered unsatisfactory for the spectrophotometric measurements, and purified 2,2,4-trimethylpentane, or iso-octane, was selected.

In purifying the carotene solution by use of the dicalcium phosphate column, the chlorophyll, if present, is adsorbed as a green band near the top of the column, the various noncarotene carotenoids appear as poorly defined bands throughout the middle of the column, and the purified carotene passes through the column. Table II compares values for β-carotene from the same samples when the purification was made by the phasic separation and when it was made by chromatographic adsorption. The methods of extraction and final measurement of the purified solutions were identical.

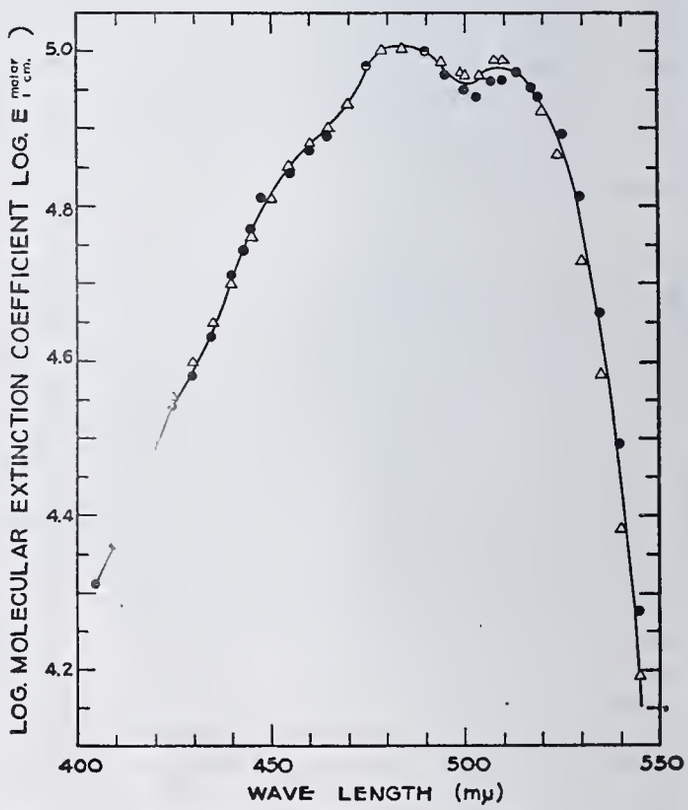

Figure 1. Molecular Extinction Curve of Pure β-Carotene in Carbon Disulfide

○ β-Carotene prepared and measured in the Southern Regional Research Laboratory
● β-Carotene prepared and measured by Smith (*23*)

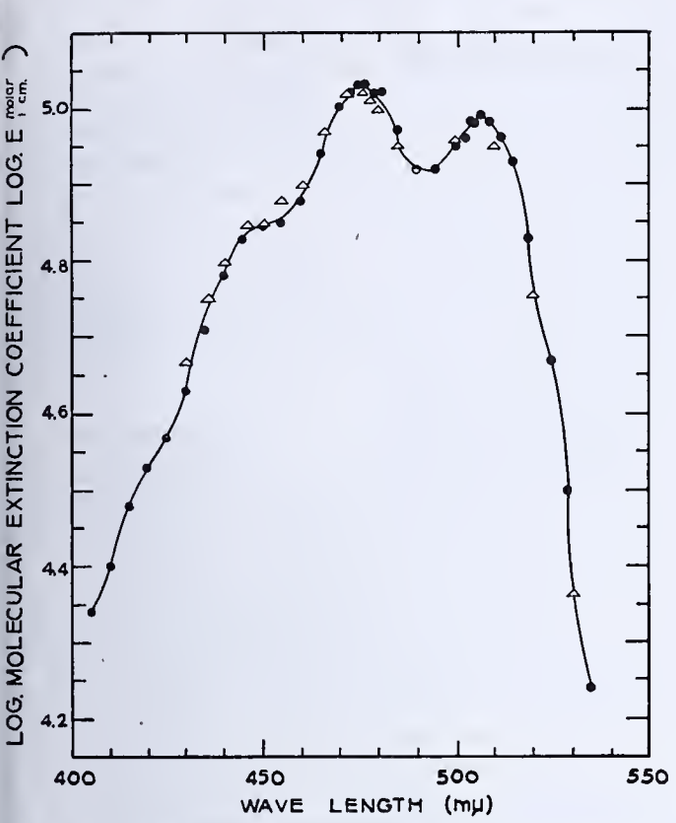

Figure 2. Molecular Extinction Curve of Pure α-Carotene in Carbon Disulfide

○ α-Carotene prepared and measured in the Southern Regional Research Laboratory
● α-Carotene prepared and measured by Smith (*23*)

ble II. Comparison of Phasic Separation and Chromatographic Column Methods of Purification of Extracted Carotene Samples

Sample No.	Carotene Content, Phasic Separation *Mg./kg.*	Carotene Content, Chromatographic Purification *Mg./kg.*
1	176.7	160.0
2	46.3	36.3
3	20.5	15.5
4	50.0	40.0
5	65.0	53.8
6	450.0	435.0
7	950.0	900.0

SPECTROPHOTOMETRIC MEASUREMENT OF PURIFIED CAROTENE

Spectrophotometric methods, when properly used for the quantative evaluation of the concentration of a substance in solu-on, are more sensitive, more precise, and more accurate than sual or photoelectric methods. In using spectrophotometric ethods, certain precautions must be observed rigidly. The ectrophotometer must provide adequate spectral isolation and e capable of permitting reproducible and accurate measurements extinction coefficients. The solutions must be purified of subances having interfering absorption. The solvent selected and ed must be reproducible in purity and composition. The exnction coefficients, at appropriately selected wave-length posions of characteristic adsorption, must be carefully determined om the most highly purified standards obtainable.

Calibration of Spectrophotometer. The Cenco-Sheard ectrophotelometer used in these investigations was equipped ith an aluminum replica grating to provide a smooth over-all esponse. The wave-length drum readings were calibrated by isual observation of the atomic lines from an A-H-4 mercury apor lamp, and adjustments made in the positioning of the grating until agreement between the drum readings and true wave ngth of the mercury lines averaged within 0.3 to 0.4 mμ. Further checks which assured satisfactory performance of the instrument included a comparison of the absorption curve of an equimolecular mixture of potassium chromate and cupric sulfate entahydrate, the solution proposed by Weigert (*25*) for calibration of spectrophotometers, with the curves obtained by Weigert (*25*) and by Smith (*23*) and a comparison of the transmission curve of a Corning didymium filter glass, CG No. 512, at a constant effective slit width of 5 mμ, with the transmission curve of the identical glass obtained from a General Electric recording spectrophotometer at the National Bureau of Standards.

Test of linearity of over-all response was made by plotting brightness measurements in foot-candles, as obtained by use of a small spot photometer, against intensities as read from the galvanometer of the spectrophotometer. Test for constancy of the intensity ratios was made by measuring the initial intensity and the transmitted intensity of a neutral glass filter over a series of varying original intensity levels. Both tests show that the response of the instrument is strictly linear and reliable only when galvanometer deflections are more than 2 cm. Consequently, in all studies reported in this paper the carotene samples were diluted until suitably large deflections for accurate measurement were obtained. A suitable concentration is about 2 mg. per liter, when 1-cm. absorption cells are used.

Purification of Solvents. The hydrocarbon and petroleum ether solvents were purified by adsorbent methods employing chromatographic columns of silica gel as described by Graff, O'Connor, and Skau (*6*). The carbon disulfide was purified by the methods described by Obach (*17*) and by Sameshima (*20*).

Purification of Crystalline Carotene. The pure α- and β-carotenes obtained from the S.M.A. Corporation were repurified by repeatedly subjecting petroleum ether solutions to chromatographic purification through aluminum oxide columns, recrystallizing from 95% ethyl alcohol and dioxane, and carefully drying over phosphorus pentoxide. These procedures were continued until no increase in the magnitude of the absorption coefficients was observed and the measured values agreed with those reported in the literature. The finally purified carotenes gave single-banded adsorption on alumina columns and no adsorption on dicalcium phosphate columns.

In Figure 1 the logarithm of the molecular extinction coefficient (extinction coefficient times molecular weight, or logarithm $E_{1\text{ cm.}}^{\text{molar}}$) is plotted against the wave length in millimicrons. This figure shows the excellent agreement between the data for the β-carotene purified in this manner and those obtained by Smith (*23*). Figure 2 shows similar agreement for α-carotene, which was purified in an identical manner.

Solvent Effects. The widely used procedure of Peterson, Hughes, and Freeman (*18*) employs a spectrophotometer for the final measurement of the carotene solution. Optical density measurements are made at 450, 470, and 480 mμ. Through the use of recommended extinction coefficients given in Table III for a choice of three solvents, these absorption measurements are converted into concentration of carotene by use of the Beer-Lambert law. It has been observed by others (*10*, *15*) that this method does not yield reproducible results, particularly when studied in collaborative work. The same value for the carotene content is not obtained when measurements are made at the specified wave lengths, with the specified solvents. With carefully purified carotenes the extinction coefficients are considerably different from those recommended. Spectrophotometric curves of the purified carotene in any of the recommended solvents (Table III) show no apparent reason for the selection of the particular wave lengths for measurement of the concentration. It is reasonable to postulate, and will be proved by experiments, that these discrepancies are due primarily to a shift in characteristic absorption occasioned by differences in solvents actually used and not due to instrumentation errors nor to impurities.

Table III. Extinction Coefficients for β-Carotene (*18*)

Wave Length	80% Ethyl Ether 20% Ethyl Alcohol Miller (*12*)	Petroleum Ether (B.p. 40–60° C.), Seibert[a]	Skellysolve B (B.p. 60–70°C.), Authors[b]
4500	247	243	238
4550	243	231	227
4700	210	207	200
4800	221	212	212

[a] Coefficients obtained from H. F. Seibert, S.M.A. Corporation, Cleveland, Ohio, and checked by authors.
[b] Skellysolve B, a special commercial grade of petroleum ether, can be successfully substituted for petroleum ether (b.p. 40° to 60° C.) in the modified method described.

These discrepancies are not due to instrument performance or calibration, as they are observed after tests described above have shown the performance of the spectrophotometer used to be satisfactory.

The influence of impurities in the extracted carotene in causing the shift of wave-length positions of characteristic absorption can be shown to be insignificantly small. The extracted carotene purified by the adsorption method described and by the phasic separation and the crystalline β-carotene repurified by the exhaustive process outlined, all contain varying amounts of impurities. The crystalline β-carotene is probably very pure (compare Figure 1). The extracted carotene purified by adsorption contains only isomers of β-carotene, while that purified by phasic separation undoubtedly contains other noncarotene carotenoids as well. When these three preparations are dissolved in either of the hyrocarbon solvents recommended by Peterson, Hughes, and Freeman (*18*) identical absorption curves, as regards the wave-length position of characteristic absorption, are obtained. When they are dissolved in the second hydrocarbon solvent again identical absorption curves are obtained, which differ, however, considerably from the first set of curves. The fact that the three preparations give identical curves in the same solvent shows that impurities are not responsible for the shift of characteristic absorption. The fact that different curves are obtained in the two hydrocarbon solvents is indicative of a solvent effect. Furthermore, the repurified carotene in the hydrocarbon solvent does not give a curve which agrees with that given by Peterson, Hughes, and Payne (*19*). However, the same carotene in carbon disulfide gives a curve which agrees exactly with the one observed by Smith (*23*). This is again an indication of solvent effect.

Moore (*13*) has shown that, by careful chromatographic separation on dicalcium phosphate columns, a number of unnamed noncarotene carotenoids can be removed from the β-carotene extracted by the phasic separation. These experiments have been confirmed here. As these impurities resemble α-carotene in absorption properties, the contamination of pure β-carotene with α-carotene should approximate, qualitatively at least, the effect produced by impurities. Hence, to samples of the purified β-carotene, various amounts of α-carotene were added. Table IV shows the effects of α-carotene upon the absorption of β-carotene. The maximum difference in the wave-length position of maxima and minima of the two pure pigments is about 5 mμ. The 50% alpha–50% beta mixture is found to have absorption maxima and minimum about midway between the corresponding points of the pure components, about 2 to 3 mμ from either, while 25% of alpha changed the absorption position of the pure β-carotene by only a little over 1 mμ. Consequently, the effect of less than 25% α-carotene or alpha-like absorbing impurities in β-carotene would be to produce a shift in the absorption spectra of the latter by an amount undetectable with the usual photoelectric spectrophotometer. But the difference in absorption positions between the petroleum ether solution of carotene prepared in this laboratory and that apparently used as a basis for obtaining the extinction coefficients in this solvent is about 5 mμ. The magnitude of the shift cannot be accounted for by contamination of the extracted β-carotene by small amounts of alpha-like absorbing carotenes and noncarotene carotenoid impurities.

Table IV. Effect of α-Carotene upon the Wave-Length Position of the Absorption of β-Carotene in Iso-octane Solution

Carotene Mixture % Beta	% Alpha	Maxima		Minimum
		mμ	mμ	mμ
100	0	450	476	465
90	10	449	475	465
75	25	448	474	464
50	50	447	474	462
25	75	446	473	462
0	100	444	473	460

It must be recognized that petroleum ethers are actually mixtures of straight-chain, iso, and cyclic hydrocarbons and, although prepared under rigidly specified procedures, may vary greatly in performance as spectrophotometric solvents. Griswold and co-workers (*7*) have shown the impossibility of preparing a truly pure hydrocarbon from such mixtures.

The importance of solvent in the study of the spectral absorption of carotenoids has been emphasized by others. Carter and Gillam (*3*) showed the wide spectral range over which the positions of maximum absorption shift with the use of different fractions of petroleum ether. Zscheile *et al.* (*30*) report inability to obtain reproducible results with petroleum ether fractions distilling over a range of 20° C.

In the present investigations a commercial petroleum ether (Skellysolve F) was subjected to a careful fractionation and the absorption of the repurified α- and β-carotenes was measured in each fraction. The wave-length positions of maxima and minimum, tabulated in Table V, show that a maximum shift of wave length positions of 6 mμ occurred between the first and fifth fractions. The variations in the composition of the hydrocarbon mixtures produced a shift in the absorption spectra of the carotenes, so that the extinction values at specified wave length are greatly affected. Particularly when measurements for the determination of total carotene are made at positions other than across the rather broad maxima, such as at the points of intersection of the alpha and beta absorption curves, which may occur on rather steep portions of the curves, a shift in the position of the absorption will produce a considerable error in the quantitative measurement. For example, from an absorption curve for β-carotene in a petroleum ether fraction, the extinction coefficient at 485 mμ is 160, at 480 mμ it is 204, while at 490 mμ it is only 120. Hence, any change in the character of the solvent such as a use of a different petroleum ether fraction which will produce a shift in the bands as much as 5 mμ, can affect the value of the extinction coefficient at the predescribed wave-length position to the extent that quantitative measurement is meaningless. Fractions which introduce smaller wave-length changes still introduce serious error in any quantitative determination.

Table V. Wave-Length Positions of Maxima and Minimum of Absorption of Carotenes in Petroleum Ether and Petroleum Ether Fractions

Petroleum Ethers and Boiling Ranges	β-Carotene Maxima	β-Carotene Minimum	α-Carotene Maxima	α-Carotene Minimum
	mμ	mμ	mμ	mμ
Skellysolve F, A.S.T.M., 30°–60° C.	448 474	464	444 470	458
Fraction 30°–35° C.	446 472	462	442 469	456
Fraction 35°–40° C.	447 474	464	444 470	458
Fraction 40°–55° C.	450 475	465	445 471	459
Fraction 55°–60° C.	452 476	466	446 472	460
Skellysolve B, A.S.T.M., 60°–70° C.	450 476	466	445 472	459

To illustrate the effect of the use of varying solvents upon the determined value of the carotene concentration, a sample of the very carefully purified and dried β-carotene was divided into numerous portions and dissolved in the petroleum ethers, Skellysolves, fractions from Skellysolve F, and other solvents for which extinction coefficients for β-carotene are available. For comparison, extinction coefficients in iso-octane (recommended later) were included. The concentration of the β-carotene in each solution was then determined. The results are tabulated in Table VI. Use of the petroleum ether solvents and the corresponding coefficients results in an error of approximately 5 to 6% of the carotene content. From the data obtained for the five fractions of Skellysolve F, with the use of the petroleum ether coefficients for Skellysolve F, it will be noted that the value for the 35° to 40° C

Table VI. Influence of Solvent on the Spectrophotometric Estimation of β-Carotene

Solvent	Source Extinction Coefficient $E^{g./l.}_{1\ cm.}$	Measured Carotene Content A: Wave length	A: Extinction coefficient	A: Carotene	B: Wave length	B: Extinction coefficient	B: Carotene	C: Wave length	C: Extinction coefficient	C: Carotene	Average carotene	Weighed Carotene Content	Discrepancy
		mμ		Mg./l.	mμ		Mg./l.	mμ		Mg./l.	Mg./l.	Mg./l.	%
Petroleum Ethers and Their Fractions with A.O.A.C. Coefficients													
Skellysolve F, A.S.T.M., 30–60° C.	(18)	450	243	1.38	470	207	1.43	480	212	1.29	1.37	1.43	4.2
Fraction 30–35° C.	(18)	450	243	1.54	470	207	1.65	480	212	1.45	1.55	1.61	3.7
Fraction 35–40° C.	(18)	450	243	1.34	470	207	1.40	480	212	1.27	1.34	1.36	1.5
Fraction 40–55° C.	(18)	450	243	1.39	470	207	1.40	480	212	1.37	1.39	1.51	8.0
Fraction 55–60° C.	(18)	450	243	1.48	470	207	1.51	480	212	1.43	1.47	1.60	8.2
Skellysolve B, A.S.T.M., 60–70° C.	(18)	450	238	1.53	470	200	1.56	480	212	1.43	1.51	1.60	5.6
	(18)	450	238	1.61	470	200	1.64	480	212	1.56	1.60	1.70	5.9
Other Solvents with Extinction Coefficients from the Literature													
Iso-octane[a]	This paper	450	245	1.99	476	219	2.00	...	...	...	1.995	1.994	0.0
Carbon disulfide[b]	(23)	484	196	2.03	513	174	2.03	...	...	...	2.030	2.034	0.2
Petroleum ether, b.p. 40–55° C.[c]	(21)	447	245	1.85	465	200	1.92	475	211	1.96	1.910	1.898	0.6
Hexane[d]	(30)	450	258	1.40	466	206	1.46	478	228	1.41	1.423	1.462	2.7
Heptane[e]	(22)	455	240	1.34	480	210	1.36	...	...	...	1.350	1.380	2.2

[a] Rohm & Haas, b.p. 98–99° C. Purified by adsorbent method (6).
[b] Purified by chemical methods (17, 20).
[c] Prepared by fractionation from Skellysolve F. Purified by adsorbent method (6).
[d] Eastman Kodak Co. synthetic, b.p. 68–69° C. Purified by adsorbent method (6).
[e] Eastman Kodak Co. synthetic, b.p. 98–98.5° C. Purified by adsorbent method (6).

action is in rather good agreement with the correct concentration. The lower boiling fraction shows a somewhat poorer agreement. With the higher boiling fractions agreement is very poor. Better values are obtained from the 55° to 60° C. fraction of Skellysolve F when the coefficients for Skellysolve B are used.

When the pure β-carotene is analyzed from solutions in iso-octane with the coefficients recommended in Table VII, in carbon disulfide with coefficients from Smith (23), and in a petroleum ether fraction from coefficients furnished by Seibert (21), very good agreement with the true concentrations is obtained. Use of Eastman Kodak Company synthetic hexane and heptane with coefficients from Zscheile (30) and Shrewsbury (22), respectively, yields carotene values somewhat too low. The hexane used by Zscheile (30) was obtained from petroleum ether and boils from 65° to 67° C. The maxima of β-carotene in this solvent were reported at 450 and 478 mμ. The hexane used in this laboratory is a synthetic product boiling from 68° to 69° C. and the β-carotene maxima were observed at 448 and 476 mμ. The heptane used here was also a synthetic product, boiling from 98° to 98.5° C., and the maxima for β-carotene were found at 453 and 478 mμ. Shrewsbury (22) does not describe his heptane but reports absorption maxima at 455 and 480 mμ. Consequently, the 2 to 3% discrepancies between the determined values of the β-carotene concentration and true values in these two solvents may be due to slight solvent effects.

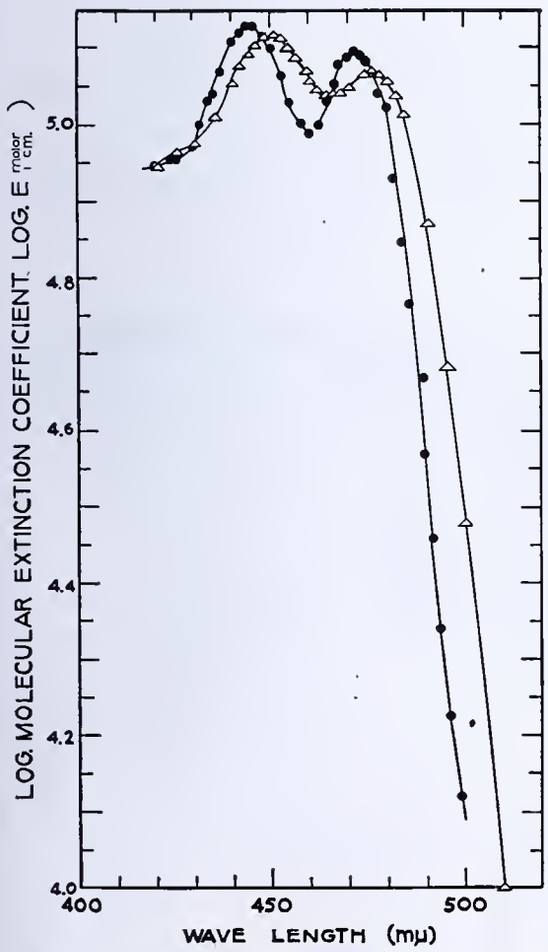

Figure 3. Molecular Extinction Curves of Pure α- and Pure β-Carotenes in 2,2,4-Trimethylpentane (Iso-octane)

● α-Carotene △ β-Carotene

The conclusion which can be obtained from the data given in Table VI is that very accurate determination of carotene by use of spectrophotometric measurements and published extinction coefficients can be obtained only if an exact description of the solvent in which they were measured is given and if that identical solvent is used.

Improved Procedure. To provide a method for the final determination of carotene by spectrophotometric measurement of the absorption of a prepared solution of the sample, and calculation of the concentration, by the use of Beer's law, from coefficients provided, a solvent which can be duplicated exactly in other laboratories must be selected. Petroleum ether fractions cannot be used with satisfaction. Griswold and his co-workers (7) have shown the presence of a large number of compounds in the petroleum ether fractions commonly used, and the impossibility of obtaining pure straight-chain hydrocarbons by fractionation. Commercially pure hexane and heptane have been suggested as solvents in the analysis for carotene (22, 30), but these are recognized as mixtures.

Iso-octane (2,2,4-trimethylpentane), the synthetic hydrocarbon introduced as a solvent in spectrophotometry by Hogness and co-workers (9) because of its ease of purification, was selected. This solvent can be obtained in a very pure state, is readily available, and its cost is not excessive. In Figure 3 the spectral absorption curves of the pure α- and β-carotenes in iso-octane are compared. Table VII is a tabulation of data from the absorption curves of the two carotenes in iso-octane solution. For the determination of β-carotene in a solution free from all interfering carotenoids, as prepared by the methods described earlier, meas-

urements at 450 and 476 mμ, peaks of the β-carotene bands, and use of the corresponding extinction coefficients, 245 and 219, respectively, permit a very accurate determination.

The method described has been used for the analyses of hundreds of samples of fresh and dehydrated sweet potatoes and of various sweet potato products. Table VIII shows representative results over the wide range of concentrations encountered in the analyses of these materials. The analysis of a carefully purified carotene by the complete method has been included in Table VI. After the spectrophotometric measurements have been completed, the carotene solutions are collected for recovery of th iso-octane by fractionation and chromatographic purificatio (*6*). In this manner the same supply of the solvent is used agai and again and the final cost of the solvent is even less than whe petroleum ether fractions, which cannot be recovered in thi manner without considerable change in character, are used.

Table VII. Extinction Coefficients for Determining Carotenes in 2,2,4-Trimethylpentane (Iso-octane)

	Carotene	Wave Length	Extinction Coefficient $E^{g./l.}_{1\ cm.}$	Absorption
		mμ		
I.	Pure α-carotene	444	252	Maximum
		473	234	Maximum
II.	Pure β-carotene	450	245	Maximum
		476	219	Maximum
III.	Total (crude) carotene	449	242	Equality
		465	202	Equality
		477	218	Equality
IV.	β-Carotene in the presence of alpha or alpha-like absorbing impurities	460	Alpha 182 Beta 214	Maximum Difference
		486	Alpha 110 Beta 176	Maximum Difference

Table VIII. Analyses of Sweet Potatoes and Various Sweet Potato Products by Proposed Method

Sample No. and Description	Carotene Content: Determination A	Determination B	Average
	Mg./kg.	*Mg./kg.*	*Mg./kg.*
Fresh sweet potato			
1	49.0	50.0	49.5
2	37.5	39.5	38.5
3	51.0	51.0	51.0
4	45.0	45.0	45.0
5	45.0	45.6	45.3
Sweet potato dried			
1	164.0	164.0	164.0
2	135.0	138.0	136.5
3	145.0	147.0	146.0
4	153.0	152.0	152.5
5	161.0	164.0	162.5
Dehydrated sweet potato (immediately after dehydration)			
1	123.0	121.0	122.0
2	110.0	112.0	111.0
3	113.0	113.0	113.0
4	85.0	87.5	86.2
5	118.0	118.0	118.0
Dehydrated sweet potato (after 30 days' storage under air)			
1	108.0	110.0	109.0
2	107.0	111.0	109.0
3	130.0	127.0	128.5
4	118.0	118.0	118.0
5	99.0	100.0	99.5
Dehydrated sweet potato (after 30 days' storage under CO_2)			
1	88.6	88.6	88.6
2	103.0	103.0	103.0
3	108.0	106.0	107.0
4	97.4	98.1	97.8
5	103.0	108.0	105.5
Sweet potato coagulum from starch plant waste water (*1*)			
1	730.0	730.0	730.0
2	1110.0	1110.0	1110.0
3	590.0	600.0	595.0
4	450.0	435.0	442.5
5	888.0	888.0	888.0
Sweet potato starch			
1	0.6	0.4	0.5
2	2.4	2.2	2.3
Sweet potato pulp dried (after removal of starch and pectin)			
1	32.0	30.2	31.1
2	22.5	22.5	22.5
3	36.7	43.3	40.0
4	50.8	50.8	50.8
5	26.2	30.0	28.1
Residue from sweet potato starch plant waste after removal of coagulum			
1	13.3	10.8	12.0
Sweet potato pulp residue			
1	49.0	50.0	49.5
2	87.5	87.5	87.5
3	85.0	85.0	85.0
4	50.8	50.8	50.8
5	90.0	100.0	95.0

The method has been shown to be applicable to the analysis of fresh and dehydrated sweet potatoes and sweet potato products. It is believed that the method is applicable to the analysis of carrots, tomatoes, and other carotene-containing materials.

SUMMARY

The three essential steps in the analysis for carotene—extractio purification, and spectrophotometric measurement—have bee studied in detail and from these studies a modified method ha been proposed for the analysis of total β-carotene in sweet pots toes and sweet potato products.

It has been shown that the inconsistencies noted in the use c published extinction coefficients for carotene in petroleum ethe fractions cannot be due solely to the presence of noncarotene im purities in the solutions and that they are due to the variabl character of these mixed solvents. The easily purified solver previously introduced into spectrophotometry, iso-octane (2,2,4 trimethylpentane), has been proposed for use in carotene analysi and extinction coefficients in this solvent have been furnished.

Use of the proposed method has been illustrated by representa tive results in the analysis of various fresh and dehydrated swee potatoes and sweet potato products.

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tudy of Millin Technique for Determination of Carbon and Hydrogen in Coal

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: Millin technique for determination of carbon and hydrogen in l has been studied. The procedures have been controlled by lysis of pure organic compounds and the technique has been nd satisfactory for many other substances that are amenable to lysis by combustion.

HE purpose of the work described in this paper has been to verify a new rapid technique first described by Millin (*3*) the macrodetermination of carbon and hydrogen in coal. e procedures used have been controlled by the analysis of pure ganic compounds, and the technique developed has been found give satisfactory results for many other substances which are enable to analysis by combustion.

Analysis for carbon and hydrogen content of coal requires from o 2 hours by the standard A.S.T.M. method (D-271-44). With e Millin procedure results for bituminous coal and anthracite available in 25 and 45 minutes, respectively. The essential ferences between the method described by Millin and that ommended by the A.S.T.M. are in the rate of oxygen flow,) ml. per minute in the former and 3 bubbles per second in the ter, and the substitution of silver turnings for a portion of the pper oxide in the combustion tube. The new procedure has en adapted for use with standard equipment and requires only e slight modification, noted above, from standard practice in arging the combustion tube.

APPARATUS

A general view of the apparatus used is shown in Figure 1; rts indicated by numbers are:

1. Needle valve and gage on supply tank of oxygen
2. Flowmeter
3. Preheating furnace filled with copper oxide wire, operating mperature 850° C.
4. Anhydrone tube for absorption of water vapor
5. Ascarite tube for absorption of carbon dioxide
6. Anhydrone–phosphorus pentoxide tube for final elimination of water vapor
7. Movable heating unit, about 13 cm. long, which serves to heat the inlet of the combustion tube and the boat containing the substance being analyzed, operating temperature 850° to 900° C.
8. Transparent section of combustion tube, 10 cm. in length
9. Movable heating unit, 33 cm. in length, used to heat the copper oxide wire and silver turnings, operating temperature 850° C.
10. Stationary furnace, 23 cm. in length, used for heating the lead chromate section, operating temperature 600° C.
11. Spun copper shield
12. Nesbitt bulb filled with Anhydrone
13. Nesbitt bulb filled with Ascarite

The fused quartz combustion tube used in this work (Figure 2) was made to specifications by the Amersil Company, Inc., Chestnut Avenue, Hillside, N. J. Its most convenient feature is the 10-cm. clear section 25 cm. from the charging end, which allows the operator to observe the combustion of the sample during the whole course of the determination. The reagents in the tube are separated in the usual manner by copper screen plugs. The movable copper oxide spiral at the entrance end is also fashioned from copper screening. The silver used is in the form of turnings tamped tightly together.

The spun copper shield over the exit end of the combustion tube serves to conduct heat from the furnace to the projecting end of the tube, thereby eliminating condensation of water vapor.

The Nesbitt bulbs used hold about 75 grams of 8 × 20 mesh Ascarite and are usable for at least 10 runs, thus allowing ample excess of reagents to ensure complete absorption of the carbon dioxide.

Figure 2 illustrates the positions and lengths of packing materials used.

PROCEDURE

With the furnaces at their operating temperatures the combustion train is tested in the usual manner until blanks indicate the apparatus is in a satisfactory condition. A gain of not more than 0.5 mg. in either absorption tube is considered satisfactory. Newly prepared tubes are conditioned by burning a small sample under normal operating procedure. After obtaining satisfactory conditions, absorption tubes 12 and 13 are attached and the boat containing the sample to be analyzed is inserted into the tube and moved to the clear section, followed by the copper oxide wire spiral. Furnace 7, which is at about 500° C., is at the extreme end of the stand. The oxygen needle valve, 1, is adjusted so that the flowmeter indicates a rate of 100 ml. per minute. The rheostat of furnace 7 is adjusted so that the unit will operate at 850° C., a temperature which it attains within a few minutes. Furnace 7 is slowly moved up to the boat and within 10 minutes will have completely covered it. After about 5 minutes both heaters 7 and 9 are pulled to the extreme end of the stand, so that furnace 7 is in its original position over the copper oxide spiral and a portion of heater 9 is over the clear section containing the charge. The

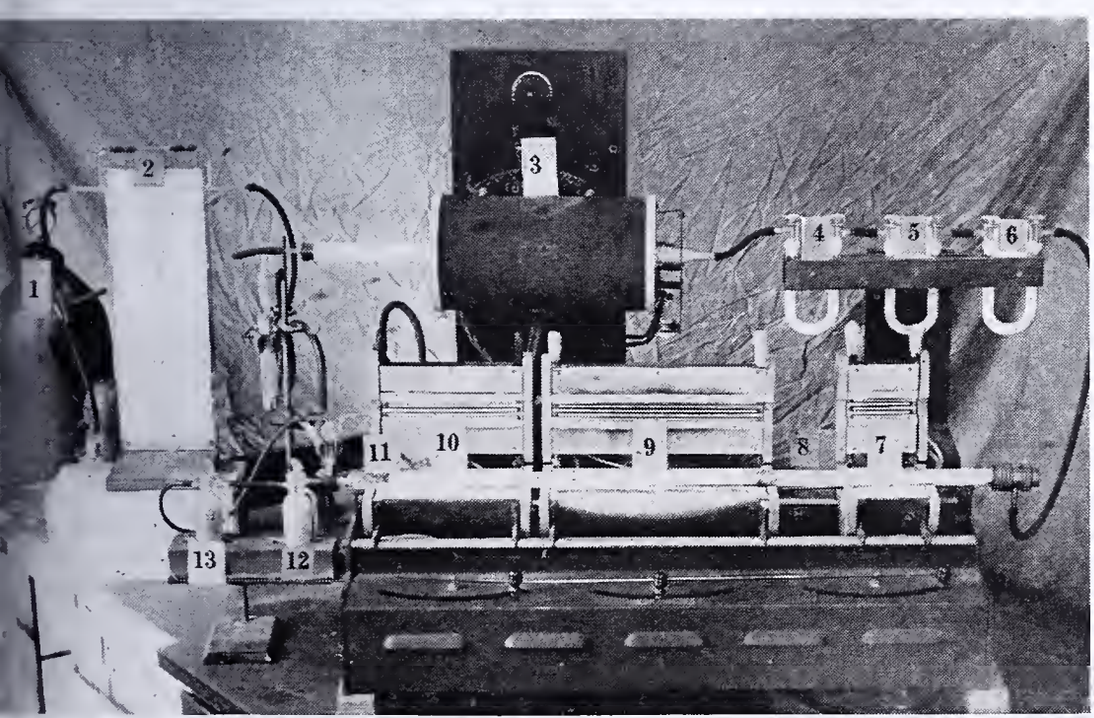

Figure 1. General View of Apparatus

rheostat on heater 7 is then adjusted to its original temperature setting, and after 5 more minutes, heater 9 is returned to its original position and the flow of oxygen is continued for another 5 minutes. At the end of this time (total of 25 minutes) the absorption tubes are shut off under pressure and then the flow of oxygen is cut off. A blowout tube, not indicated, which automatically controls the maximum pressure in the system makes this last step possible; it also prevents the manometer fluid from being forced into the train in event of misadjustment of the needle valve.

At the completion of the above steps the absorption tubes are placed near the balance and the train is ready for the next run. The tubes are allowed to cool to balance temperature, vented to the atmosphere for a few seconds while being wiped with a damp chamois skin, and then weighed, using a similar tube as counterpoise on the balance.

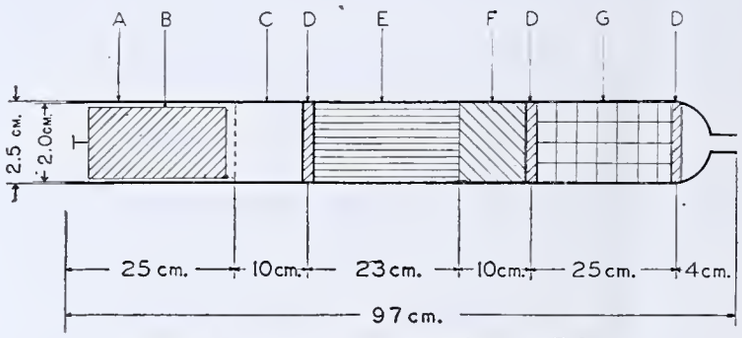

Figure 2. Fused Quartz Combustion Tube

A. Charging end
B. Removable, oxidized, copper screen spiral
C. Transparent section for combustion boat
D. Oxidized copper screen plugs
E. Cupric oxide (wire form)
F. Silver turnings
G. Lead chromate

All results for bituminous coals reported on in this paper were obtained by this procedure. In the paper by Millin (*3*) a time of 20 minutes is given for the analysis of coals; this lower rate may be due to the longer length of combustion tubing which was employed, thereby allowing the use of 35 cm. of copper oxide and 10 cm. of silver turnings in the center furnace, whereas the authors were limited, because of the standard design of the apparatus, to 23 cm. of copper oxide and 10 cm. of silver turnings. No indication was given that any of the samples analyzed were anthracites. In the case of anthracites more time was found necessary for complete combustion of the samples; hence the regular procedure was changed to allow heater 7 to be placed over the charge immediately and to remain there 10 to 15 minutes longer than usual.

The procedure was used for various other materials which, because of their greater volatility, required slight changes in technique. The increase in time required was used to volatilize the material slowly without allowing it to catch fire; the rate of oxygen flow was decreased slightly in some cases, although this was more of a conservative measure than a necessity.

In the preliminary tests to establish a minimum time for combustion, two Ascarite absorption tubes were placed in series on the train; the second in the series would show a gain if the first did not completely absorb all carbon dioxide formed during combustion. Through this procedure it was found that Fisher-type tubes, which have about one fourth the capacity for Ascarite as the Nesbitt tubes finally adopted, were too small to absorb the carbon dioxide quantitatively. To test for complete conversion of the carbon to carbon dioxide a bubbler tube containing a solution of palladous chloride (*4*) was attached to the end of the train.

RESULTS AND DISCUSSION

Table I shows typical results obtained using Bureau of Standards samples of sucrose and benzoic acid. In each case the ignition period was completed in 25 minutes and the sample tubes were removed and allowed to cool before weighings were made. In the case of benzoic acid the slightly low results for carbon may be attributed to the rapid rate at which the substance volatilized; no alteration in time of combustion was tried on this substance.

Table II gives results of analysis of a bituminous coal sample by the standard A.S.T.M. and Millin procedures. Tables III and IV give results by the Millin procedure on anthracites, bituminous coals, carburetting oil, tar, abietic acid, and liquid hydrogenation products.

Sucrose is generally used as a control standard in combustion analysis. The data in Table I show that the Millin procedure is satisfactory in the analysis of sucrose. Table II shows the results obtained with the two methods on a sample of bituminous coal which was air-dried and ground to pass a 250-micron (No. 60 sieve. All other samples of coal used were prepared in like manner. The specified permissible difference for duplicate analysis

Table I. Tests on Bureau of Standards Samples

	1	2	3	4	Theoretical
Sucrose					
C, %	42.08	42.06	42.08	41.98	42.08
H, %	6.50	6.49	6.44	6.49	6.48
Benzoic acid					
C, %	68.67	68.75	...	...	68.83
H, %	4.94	4.99	...	...	4.96

Table II. Comparative Results by A.S.T.M. and Millin Procedure on a Bituminous Coal

Method	1	2	3	Time, Min.
A.S.T.M.				
C, %	84.00	83.91	84.19	120
H, %	4.13	4.10	4.12	
Millin				
C, %	84.20	84.20	84.30	25
H, %	4.13	4.10	4.10	

Table III. Typical Results on Coals by Millin Procedure

Sample Designation	Time of Combustion	Carbon Run 1	Carbon Run 2	Carbon Difference	Hydrogen Run 1	Hydrogen Run 2	Hydrogen Difference
	Min.	%	%	%	%	%	%
Bituminous coal							
A	25	79.19	79.14	0.05	4.62	4.66	0.04
B	25	75.27	75.27	0.00	5.18	5.21	0.03
C	25	82.91	82.65	0.25	4.32	4.30	0.02
D	25	72.66	72.38	0.28	4.89	4.87	0.02
E	25	79.21	79.15	0.06	5.06	5.01	0.05
Anthracite							
F	40	81.65	81.60	0.05	2.01	2.06	0.05
G	40	79.08	79.09	0.01	2.39	2.43	0.04
H	40	80.87	81.04	0.17	2.47	2.44	0.03
I	40	84.36	84.32	0.04	2.15	2.12	0.03
J	40	85.14	85.12	0.02	2.09	2.10	0.01

Table IV. Typical Results on Organic Materials by the Millin Procedure

(Time of combustion, 75 minutes)

Sample Designation	Carbon Run 1	Carbon Run 2	Difference or Theoretical	Hydrogen Run 1	Hydrogen Run 2	Difference or Theoretical
	%	%	%	%	%	%
Carburetting oil						
K	84.92	84.70	0.22	13.82	13.80	0.02
L	85.49	85.57	0.08	13.89	13.86	0.03
M	85.44	85.24	0.20	13.71	13.76	0.05
Tar						
N	92.58	92.40	0.18	6.39	6.37	0.02
O	92.63	92.71	0.08	6.51	6.51	0.00
P	92.50	92.49	0.01	6.39	6.42	0.03
Abietic acid (recrystallized)						
Q	79.35	79.30	79.40	9.87	9.90	10.00
Liquid hydrogenation products						
R	87.68	87.73	0.05	12.14	12.12	0.02
S	88.13	88.19	0.06	11 78	11.73	0.05
T	88.68	88.67	0.01	10.95	10.99	0.04
U	88.92	88.93	0.01	10.91	10.91	0.00

Table V. Statistical Analysis for Carbon and Hydrogen

in. %	Max. %	Σn	m	s	$\Sigma n'$	m'	s'	B'	γ'
Carbon, A.S.T.M. permissible difference = 0.3%									
thracites									
.74	91.73	208	100	0.181	207	100	0.172	0.4	<100
tuminous coals									
.10	91.10	124	50	0.420	118	48	0.317	0.4	<500
Hydrogen, A.S.T.M. permissible difference = 0.07%									
thracites									
.34	3.30	211	100	0.049	211	100	0.049	0.2	<200
tuminous coals									
.34	5.78	127	50	0.143	121	48	0.088	0.8	<500

Min. Minimum value for single determination.
Max. Maximum value for single determination.
Σn. Total number of analyses reported.
m. Number of samples.
s. Standard error of difference between duplicates analyses.
$\Sigma n'$, m', s'. Values of Σn, m, and s after eliminating samples for which alyses showed evidence of gross error.
B'. Approximate measure of normality of distribution of errors after mination of gross errors.
γ'. Calculated frequency of exceeding A.S.T.M. "permissible" difference tween duplicate determinations per thousand.
<10 = 0 to 9.9, <20 = 10 to 19.9, <100 = 50 to 9.99, <200 = 100 to 199, etc.

ccording to A.S.T.M. standards on coal and coke (*1*), is 0.30% or carbon and 0.07% for hydrogen. That the duplicates obtained y this new procedure fall well within these limits is shown by the ata of Table III, where a maximum difference of 0.28% is given or carbon and of 0.05% for hydrogen. Duplicate results that ll within the specified tolerances are generally obtained from wo analyses, a third analysis being necessary only occasionally. n this respect it is usually the carbon value that is outside the olerance and seldom the hydrogen.

Several other types of materials were analyzed (Table IV). t was found necessary, owing to the greater volatility of these ubstances, to lengthen the time of combustion to 75 minutes. side from this increase in time, which is considerably shorter han that used in the older method, the method works consistently ell. The abietic acid analyzed was allowed to stand for some ime before an analysis was made on it and may have been of uestionable purity. The liquid hydrogenation products may ave contained traces of water. They were separated by dis illation and dried over phosphorus pentoxide and were expected o be pure hydrocarbons.

Table V summarizes the results obtained from a statistical nalysis of 100 sets of values for percentages of carbon and hydrogen in 100 different samples of anthracite coals. These data were calculated in a manner identical to that used by Lowry and Junge (*2*). For comparison purposes data submitted by this laboratory and reported by Lowry and Junge (*2*) for 50 sets of values on 50 different samples of bituminous coals are included. An examination of Table V shows that values of B' in every case are less than unity; the data themselves do not give an indication of departure from normality.

The values of the frequency, γ', show a decided improvement in the case of both carbon and hydrogen, as do the values of s and s'. Analytical procedures are frequently controlled by the use of statistical methods, to give the equivalent of a "reasonable" permissible difference, which may be defined as that difference between duplicate determinations which would not require a third determination more than 50 times in 1000. A "reasonable" permissible difference equal to 1.96 s' would yield a value of $\gamma' = 50$.

In comparing the two methods it appears that a "reasonable" permissible difference for carbon by the Millin procedure would be calculated from the data on anthracites to be 0.34% and by the A.S.T.M. method on bituminous coals to be 0.62%, and similarly for hydrogen 0.10 and 0.17%.

Inasmuch as the data reported were obtained in small groups (never more than ten samples were run consecutively) and the whole group was collected over a period of 3 years, with four different operators during that period, it seems reasonable that for routine operation the limits should not be expanded. The following quotation from Selvig's comment (*5*) on the paper of Lowry and Junge (*2*) seems appropriate:

> It is not surprising that difficulties were experienced in the ultimate analysis in determining carbon and hydrogen, as this analysis requires a higher degree of manipulative skill and good condition of the combustion train, both of which are difficult to attain if determinations are made at irregular intervals. Here possibly it would be better not to raise the permissible difference between duplicate determinations but to make multiplicate rather than duplicate determinations to obtain the required precision.

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Estimation of Oxygen in Inert "Oxygen-Free" Atmospheres

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A GROWING number of industrial processes require atmospheres in which the oxygen content is far below 0.01% by volume. An analytical method capable of dealing with these minute concentrations of oxygen in atmospheres inert to the reagents employed is described below.

Oxygen at concentrations near 0.0001% in 1 liter of gas at room temperature and atmospheric pressure exerts a pressure below 1 micron and weighs about 1 microgram. The successful analysis of such a sample could be accomplished, but only by taking extreme precautions to eliminate measurable contamination before liberation of iodine and by determining this iodine in a solution that could be exposed almost with impunity to the atmosphere.

CHOICE OF METHOD

Of the chemical methods for estimating oxygen, the two most promising for further refinement seemed those involving the oxidation of ferrous or of manganous compounds in alkaline media.

The use of ferrous salts for the determination of oxygen was originated by Mohr (*2, 8*) and developed further by Shaw (*11*), who determined the ferric ion colorimetrically with thiocyanate in acid solution, the blank being near 0.008% oxygen. The portion of this blank traceable to the ferrous ammonium sulfate was practically eliminated in this laboratory by leaving acid ferrous ammonium sulfate solutions overnight on the steam bath in contact with pure aluminum. Unfortunately, the oxidation, by the laboratory air, of ferrous ion in acid solutions could not be sufficiently reduced.

The use of manganous hydroxide and an iodide to determine oxygen dissolved in water was developed by Winkler (*12*) in a classic investigation. In this sensitive method, the oxidized manganous hydroxide liberates upon acidification an amount of iodine equivalent to the dissolved oxygen initially present. Various investigators (*1, 3, 4, 6, 9, 10*) have adapted this scheme to the determination of oxygen in gases. Hand (*4*) demonstrated

A practical, sensitive, and reasonably reliable method, based upon the liberation of iodine by oxidized manganous hydroxide, has been developed for the determination of oxygen at concentrations above 0.0001% by volume in a liter of gas at atmospheric pressure; 0.01% is perhaps the concentration most convenient for the method. The decreasing reactivity of manganous hydroxide as its surface becomes oxidized sets the useful upper limit for the method near 0.1% oxygen. The final iodine determination, whether spectrophotometric (0.001% oxygen or less) or by titration with thiosulfate, must be carried out in solutions stable toward air.

that great sensitivity could be achieved in this way. His method suffers from the drawback that the sample must be introduced into a complicated apparatus that has been previously evacuated, and his preparation of standards is open to objection.

STABILITY OF STANDARD IODINE SOLUTIONS

Standard solutions containing iodine in microgram amounts, with and without added starch, were investigated on the G.E. recording spectrophotometer (*7*) in order to find conditions under which handling in contact with air would not cause appreciable oxidation of iodide.

The first standards were prepared according to Hand (*4*) by adding known amounts of permanganate ion to acid iodide solutions. After the permanganate had reacted, these standards continued to deepen rapidly in color. This instability ("after-bluing" or "oxygen error"), which is well known in iodometry, showed these solutions to be useless for the authors' purposes.

Satisfactory standards were finally made from known permanganate solutions.

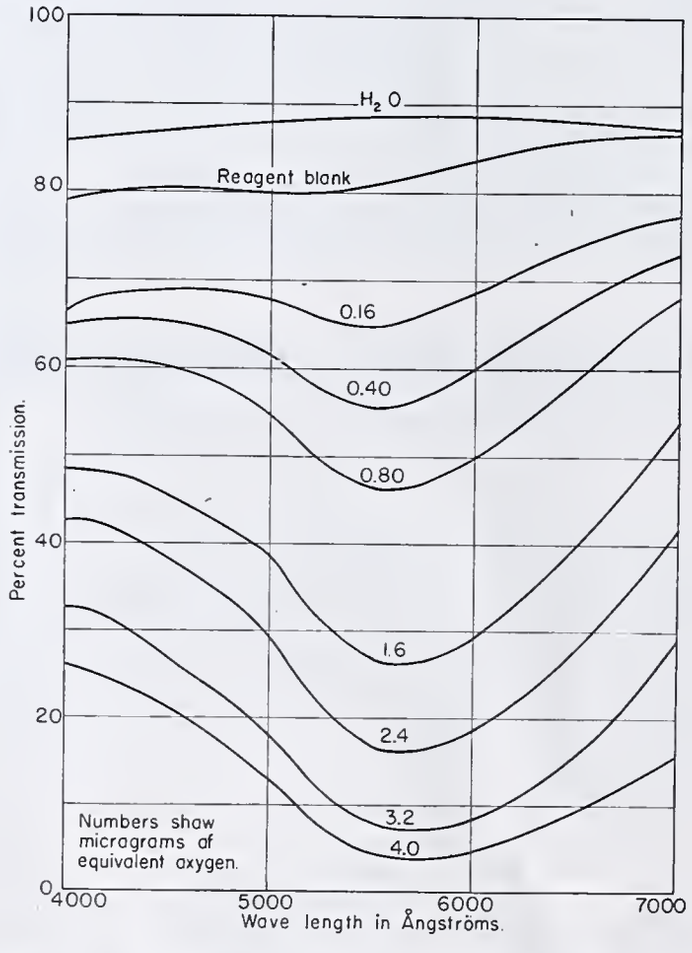

Figure 1. Transmittance Curves for Iodine Standards with 1 Cc. of Starch

The various reagents were added to glass-stoppered gradua in the order: 2 cc. of 1 *N* sodium hydroxide–0.1 *M* potassiu iodide solution; 1 cc. of 3 *N* sulfuric acid; 0.5 cc. of 0.5 *M* ma ganous chloride; 0.001 *N* or 0.0001 *N* permanganate soluti freshly prepared by dilution; 2 cc. of 0.6 *M* sodium bicarbona 1% starch, 0.1 or 1.0 cc.; finally, boiled distilled water to volume of 27 cc. The bicarbonate neutralizes acid and provi an inert atmosphere. It was blown in to ensure rapid action a to prevent the undesired precipitation of manganous carbona This addition of bicarbonate leaves the solution weakly ac more was subsequently added dropwise to raise the pH to abo 5.

In preparing standards from iodine solutions by the foregoi procedure, the permanganate was omitted, and the known iodi solution was added after the bicarbonate.

A representative family of absorption curves for the starc iodine complex thus prepared is shown in Figure 1. Even w scrupulous cleanliness, a little color, representing usually a f tenths microgram of oxygen, developed when neither perma ganate nor iodine was added; the "reagent blank" curve Figure 1 is an example. The wave length for maximum abso tion shifts toward the red as the concentration of the starc iodine complex increases. In making the Beer's law calculatio I_0 for the reagent blank and I for the standard were taken at t wave length of maximum absorption.

The stability of standard solutions made according to the fo going method was tested on the spectrophotometer. The curve Figure 2, for example, was obtained by having the instrume record continuously for 26 minutes the transmittance of a star ard containing the blue starch-iodine complex at a concentrati equivalent to about 0.4 microgram of oxygen. (The instrume draws one complete curve every 3 minutes.) Standards co taining starch cannot be kept for more than a few hours befo blue particles begin to separate; this separation may have beg in the test of Figure 2, for the transmittance actually increased little during the test. In any case, there was no indication appreciable "oxygen error" in this or similar experiments.

Beer's law plots are shown in Figure 3 for three sets of standar An insufficiency of starch appears to be responsible for the re tively low light absorption at the higher concentrations (compa curves A and B). In the most dilute range, the permangana standards (curve C) absorbed less light than corresponding on prepared with iodine (curve B), possibly because the reaction w quenched too soon in the attempt to reduce the risk of oxidati of iodide by air.

The spectrophotometric work thus proved that unwant iodine formation could be made negligible. It also proved th the estimation of oxygen at concentrations below about 0.001 in 1 liter of gas can be carried out spectrophotometrically on t equivalent amount of iodine.

METHOD FOR OXYGEN

Reagents. A solution 1 *N* in sodium hydroxide and 0.1 in potassium iodide; 3 *N* sulfuric acid; 0.5 *M* manganous chlorid slightly acid; 0.6 *M* sodium bicarbonate; 0.002 *N* (or anoth suitable concentration) sodium thiosulfate stabilized with sodiu carbonate, freshly prepared by dilution; an inert gas containi no oxygen detectable by the present method.

The "oxygen-free" gas in this work was hydrogen that had be passed slowly (110 cc. per minute) in an all-glass system ov copper at 600° C. and then over sodium hydroxide pellets to r move hydrogen sulfide. A 6-liter bulb inserted after the sodiu hydroxide served as a reservoir, so that 1 liter of hydrogen cou be withdrawn rather suddenly without diminishing the effectiv ness of the purifier.

All solutions used for oxygen determinations were kept in g washing bottles through which "oxygen-free" hydrogen w bubbled continuously. Rubber tubing used in connecting the and other vessels to the purification system was kept at a mir mum.

The purity of the manganous chloride, which must be low iron, is especially important; but all chemicals should be of hig quality. The fact that repeated analyses of the purified hydrog showed no detectable iodine proved that the reagents and th

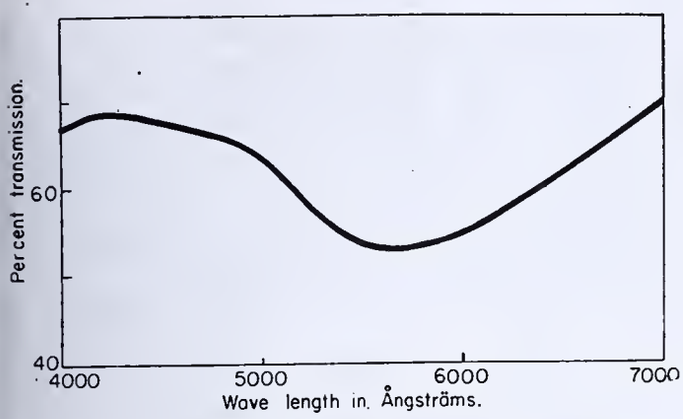

Figure 2. Stability Test on Permanganate Standard

Curve retraced continuously over 26-minute period in spectrophotometer

ıanipulation were good enough to keep oxygen or oxygenlike npurities below 1 microgram of equivalent oxygen.

Apparatus and Manipulation. The 1-liter reaction vessel Figure 4) must be dry and clean; drops of a liquid that has been ı contact with air must be avoided. Whenever glass apparatus pipet included) has been in contact with manganous solutions nd with oxygen, films of oxidized manganous compounds may be resent; such films can usually be removed with strong hydrohloric acid. After a determination in which the oxygen content xceeded 0.1%, however, sodium sulfite was added along with the cid in the cleaning process. Boiled water and acetone were used or rinsing. Stopcocks were carefully greased and cleaned between eterminations. Only vacuum-tested stopcocks were used, and hese had occasionally to be changed, probably because of attack y the alkaline solutions.

The gas sample was usually obtained by streaming the gas to e analyzed for several hours through the reaction vessel. After he two stopcocks nearest the spherical bulb had been closed, the ection below the lower stopcock was filled with water and tightly orked.

The solutions were introduced in the following order from the ylindrical reservoir into the spherical bulb: 10 cc. of sodium hydroxide–potassium iodide solution; 5 cc. of manganous chloride; 8.5 cc. of sulfuric acid. No matter what the solution, it was transferred by means of a pipet from the gas washing bottle into the clean, dry cylindrical reservoir through the female ground joint. The apparatus was then connected by means of the upper male ground joint (Figure 4) to the 6-liter bulb in the oxygen-free hydrogen train. The outlet from the cylindrical reservoir was connected to a bubbling bottle filled with sulfuric acid. The "oxygen-free" hydrogen was bubbled for 10 minutes through the solution in the reservoir; then the outlet was connected to the rough vacuum, whereupon the gas space in the reservoir was twice evacuated and refilled with the hydrogen. With all four stopcocks closed, the reaction vessel was disconnected, and the solution was transferred to the bulb by cooling the latter under the tap, by opening the necessary stopcock, and by careful shaking. The cylindrical reservoir was next cleaned with strong hydrochloric acid, and rinsed with boiled water. The stopcock and joints affected were regreased, and the reaction vessel was reconnected to the hydrogen train to receive the next solution.

After the first two solutions had been introduced into the bulb, the reservoir and the attached tubes were filled with boiled distilled water, and the two upper stopcocks were closed. The vessel was then rotated by hand to form manganous hydroxide and to distribute it uniformly. It was next wrapped in a towel and strapped centrally to the table of a rotary shaker and shaken (usually for 2 hours) at a rate near 165 cycles per minute. Finally the sulfuric acid was transferred into the bulb in the manner described above.

The acid iodine solution was then drained out of the reaction vessel, and the pH raised rapidly to a value between 4 and 6 by adding sodium bicarbonate solution. Such solutions in glass-stoppered flasks could be kept in the dark without gaining appreciably in iodine content, which is not surprising since their compositions approximated those of the stable standards discussed above. Usually the iodine was titrated on an aliquot portion of the solution with thiosulfate, 0.002 *N* or stronger, 1 cc. of starch having been added.

Sensitivity and Reliability. The work of Winkler (*12*) and subsequent experience with his method leave no doubt that the oxidation of manganous hydroxide and the subsequent liberation of iodine affords the best chemical method for estimating oxygen dissolved in water. On this basis, the present method may be considered reliable, provided that (1) the blank is negligible, (2) the time of shaking is long enough to reduce all the oxygen, and (3) there is negligible "oxygen error" when the final solutions are handled in contact with air. Proof of the last point was given above.

When "oxygen-free" hydrogen was analyzed, no oxygen could be detected by the present method, the blank being considerably less than 1 microgram of oxygen. A similar result was obtained on the evacuated reaction vessel when no leaks occurred. This proves a negligible blank and also shows that oxygen adsorbed on the walls may be neglected. The last conclusion is in accord with the literature (*5*).

What constitutes a sufficiently long time of shaking is more difficult to establish, owing to the decrease in the reactivity of manganous hydroxide as its surface becomes oxidized. This decrease in reactivity was demonstrated in two series of experiments (Tables I and II).

In the experiments of Table I, the reaction vessel was exhausted to the pressure corresponding to the per cent residual oxygen, and immediately filled to atmospheric pressure with "oxygen-free" hydrogen. Oxygen determinations gave the results in the last

Table I. Time of Shaking at Different Concentrations of Residual Oxygen

No.	Time of Shaking *Hours*	Residual Oxygen *% by volume*	Oxygen Found *% by volume*
1	1	0.0013	0.0015
2	1	0.028	0.020
3	1	0.028	0.019
4	2.5	0.028	0.028

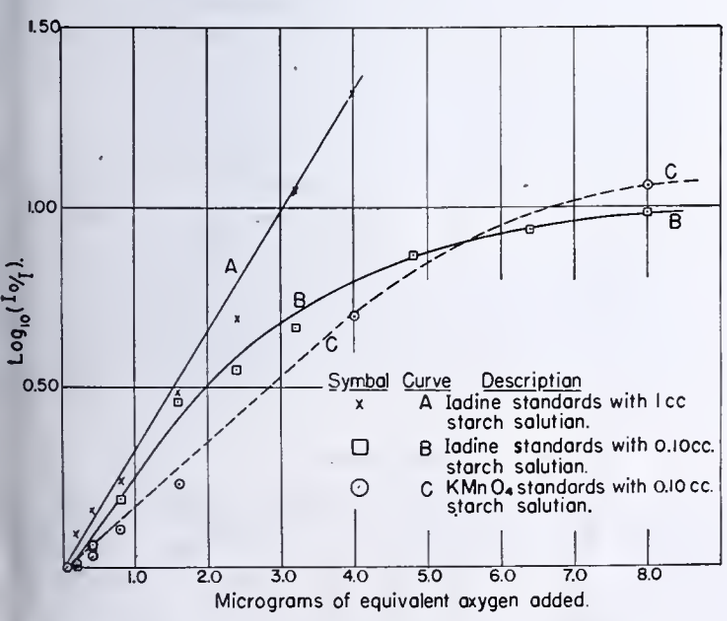

Figure 3. Beer's Law Curves for Iodine and Permanganate Standards

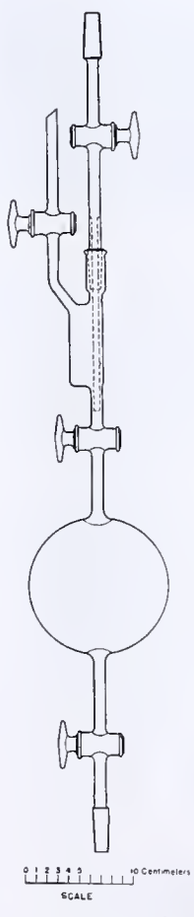

Figure 4. Reaction Bulb for Oxygen Determinations

Table II. Results on Nitrogen at Various Partial Pressures

No.	Time of Shaking *Hours*	Oxygen in Reaction Vessel *% by volume*	Oxygen in Nitrogen *% by volume*
1	2.5	0.45	0.22
2	2	0.012	0.50
3	2	0.030	0.52

column. It is obvious that the requisite time of shaking increases with the initial oxygen concentration.

In the experiments of Table II, varying pressures of nitrogen that contained 0.45 ± 0.05% oxygen according to a careful Orsat analysis were left in the reaction vessel and supplemented with "oxygen-free" hydrogen as above. The percentages in the last column are to be compared with 0.45%; the others are the initial concentrations in the reaction vessel.

The results cited set the upper limit of usefulness for the method near 0.1%; more concentrated samples should be diluted if they must be analyzed in this way. The useful lower limit is near 0.0001%; 0.01% is perhaps the most favorable concentration. The color change in the manganous hydroxide during the analysis is a rough guide to the oxygen content of the sample.

It has not been possible to fix the precision of the method which will obviously be lowest near 0.0001% oxygen. Duplicate determinations on nitrogen known to be very low in oxygen each gave 0.0004%. Certainly the precision is not so high as Winkler's (*12*); the reader may form his own estimate from Tables I and II.

The method is unreliable at pressures much below atmospheric. Numerous failures were encountered in attempts to establish blanks by analyzing for oxygen in the evacuated reaction vessels, and there is no doubt that leaks were responsible.

The time required for a determination lies between 4 and 8 hours.

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Volumetric Determination of Aluminum

Applied to Analyses of Silica-Alumina Catalysts

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A volumetric method for the determination of aluminum in silica-alumina catalysts is described, which is more rapid and more accurate than conventional gravimetric methods. The aluminum sulfate solution, containing tartrate, is neutralized with lithium hydroxide, and an excess of potassium fluoride is added. The potassium hydroxide released by the formation of the aluminum complex is titrated with hydrochloric acid.

ACTIVITY studies of silica-alumina catalysts recently carried out at these laboratories necessitated a large number of aluminum determinations. It was important that these determinations be carried out as rapidly as possible and still retain a high degree of accuracy, and that the method employed be applicable in the presence of moderate amounts of other substances, such as iron, magnesium, and alkalies.

The conventional method involving hydrolysis of the aluminum with ammonia, filtering, igniting, and weighing, was not only time-consuming but also subject to interference by other hydrolyzable materials present. A method often used is one in which the residue, after treatment with hydrofluoric acid and ignition, is calculated as alumina. This procedure is also time-consuming because of difficulty in igniting the residue to a constant weight, and alumina values thus obtained include all other metals present in the sample.

Several volumetric procedures for the determination of aluminum (*1–6*) are based on the titration of either the acid liberated on addition of sodium tartrate (or citrate) or the hydroxide liberated on addition of potassium fluoride.

The most generally applicable of the volumetric methods for aluminum appears to be the one recently described by Snyder (*4*). In this procedure the aluminum salt is neutralized to a phenolphthalein end point with barium hydroxide in the presence of sodium potassium tartrate. An excess of potassium fluoride is then added to release potassium hydroxide, which is titrated with standard acid.

$$Al(OH)_3 + 6KF \rightarrow AlF_3.3KF + 3KOH$$

As stated by Snyder, this method cannot be applied in the presence of sulfate because of the precipitation of barium sulfate. In the analysis of silica-alumina catalysts large amounts of sulfate are necessarily present because of the methods required for dissolving the samples. The purpose of this paper is to describe the modifications that have been made in the method developed by Snyder which make it suitable for the rapid and accurate determination of aluminum in silica-alumina catalysts.

Since barium hydroxide could not be used for the neutralization of the aluminum salt, another base had to be found. The fact reported by Snyder that sodium and potassium hydroxides give erratic and inaccurate results was confirmed. It was found that lithium hydroxide has none of the undesirable characteristics of sodium and potassium hydroxides and does not form an insoluble sulfate as does the barium hydroxide. It was decided from a study of the potentiometric titration curves, and confirmed by experience, that thymolphthalein is a more suitable indicator than phenolphthalein.

EXPERIMENTAL

APPARATUS AND REAGENTS. Mechanical stirrer. Platinum dish, 100 ml. Potassium fluoride, 30% solution neutralized to phenolphthalein and stored in a wax-lined bottle. Sodium tartrate, 25% solution. Thymolphthalein, 0.1% in ethanol. Aluminum sulfate solution, 0.5 *N*, prepared by dissolving 55.535 grams of aluminum sulfate octadecahydrate in 1 liter of water. The alumina content of the aluminum sulfate was found by igniting samples at 2000° F. and weighing. Hydrochloric acid

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.5 N, standardized against the aluminum sulfate solution. ithium hydroxide, 0.5 N, standardized against the 0.5 N ydrochloric acid. (Ratio of milliliters of hydrochloric acid to illiliters of lithium hydroxide is equal to R.) All reagents ere C.P. grade and were used without further purification.

PROCEDURE. Weigh a 1.0 to 1.5-gram sample into a platinum ish and ignite to remove carbon and ammonium salts. Cool, oisten the sample with water, and add 10 ml. of hydrochloric cid. Cautiously add hydrofluoric acid until the sample is completely dissolved. Evaporate the solution to dryness and ignite t red heat for a few seconds. Fuse the residue with approximately 3 grams of anhydrous potassium bisulfate over a low flame ntil no undissolved particles remain. Cool, dissolve the fusion n 80 ml. of water, and transfer to a 400-ml. tall-form beaker.

Place the beaker containing the dissolved sample under the echanical stirrer and titrate with the lithium hydroxide. When ost of the free acid is neutralized, as evidenced by the appearnce of traces of precipitate in the solution, add 6 to 8 drops of hymolphthalein indicator and 25 ml. of 25% sodium tartrate. f the solution contains considerable magnesium or lead or more han 0.3 gram of aluminum oxide, add 50 ml. of the sodium tarrate. Continue the titration until appearance of a blue color, ermanent for 30 seconds, and add 0.1 to 0.2-ml. excess of lithium ydroxide. Back-titrate with the 0.5 N hydrochloric acid until he blue color just disappears. Record the readings of the hydrohloric acid and lithium hydroxide burets as A_1 and B_1, respecively. Add 25 ml. of 30% potassium fluoride. The solution ecomes blue immediately because of the liberation of hydroxyl ons. Titrate with the hydrochloric acid until the blue color lisappears and add 1-ml. excess. Back-titrate with the lithium ydroxide until the blue color reappears. Record the readings of he hydrochloric acid and lithium hydroxide burets as A_2 and B_2, espectively. The back-titrations are necessary to ensure sharp, ermanent end points.

CALCULATIONS.

$$\text{Weight \% of } Al_2O_3 = \frac{[A - (B \times R)] \times N \times 0.017 \times 100}{\text{weight of sample}}$$

where A = $A_2 - A_1$ (volume of HCl)
B = $B_2 - B_1$ (volume of LiOH)
N = normality of HCl
R = ratio $\frac{\text{ml. of HCl}}{\text{ml. of LiOH}}$

TITRATION OF KNOWN SOLUTIONS. The reliability of the method was tested by titrating a number of solutions of known aluminum content. The solutions were prepared by dissolving aluminum wire in sulfuric acid or sodium hydroxide and by dissolving aluminum sulfate in water. The composition of the solution prepared from aluminum sulfate was found by evaporating aliquots to dryness, igniting to Al_2O_3 at 2000° F., and weighing. The results of these analyses are listed in Table I.

Table I. Analysis of Known Aluminum Solutions

Solution	Prepared from	Al_2O_3 Added, *Gram*	Al_2O_3 Found, *Gram*
1	Al wire	0.0638	0.0639
2	Al wire	0.0638	0.0642
3	Al wire	0.0830	0.0832
4	$Al_2(SO_4)_3$	0.1532	0.1532
5	$Al_2(SO_4)_3$	0.1532	0.1530
6	$Al_2(SO_4)_3$	0.1532	0.1534

INTERFERENCES. A study was made of the effects of the ions that may sometimes be present in silica-alumina catalysts. The impurities were added so as to be approximately equivalent to 5% of the material in a sample. Of the ions tested, only ammonium, tin, and manganese caused significant interference. Larger amounts of the colored ions, such as copper, chromium, and nickel, obscure the end points. The results of the interference studies are shown in Table II. It was found that a fivefold excess of iron and magnesia does not interfere if sufficient tartrate is added.

ANALYSIS OF SAMPLES. Several synthetic samples were prepared by adding 15.32% aluminum oxide to pure silica. Repeated analyses of these samples are shown in Table III.

Table II. Effect of Interfering Substances

Impurity	Al Added, *Gram*	Al Found, *Gram*	Difference, *Gram*
Iron	0.0638	0.0638	0.0000
	0.0859	0.0856	−0.0003
Nickel	0.0859	0.0856	−0.0003
	0.1429	0.1420	−0.0009
Copper	0.0859	0.0861	+0.0002
Magnesium	0.0859	0.0862	+0.0003
	0.1429	0.1432	+0.0003
Calcium	0.0859	0.0864	+0.0005
	0.1429	0.1431	+0.0002
Sodium	0.0859	0.0850	−0.0009
	0.1429	0.1426	−0.0003
Potassium	0.0638	0.0638	0.0000
	0.1429	0.1421	−0.0008
Lead	0.0859	0.0846	−0.0013
Chromium	0.0859	0.0852	−0.0007
Manganese	0.1429	Color obscures end point	
Ammonium	0.1429	No end point	
Tin	0.1429	0.1321	−0.0108
Phosphate	0.1429	0.1438	+0.0009

Table III. Analysis of Synthetic Samples

Sample	Al_2O_3 Added, %	Al_2O_3 Found, %
1	15.32	15.30
2	15.32	15.39
3	15.32	15.34
4	15.32	15.32
5	15.32	15.31

Table IV. Analysis of Routine Laboratory Samples

Sample	Type of Catalyst	Al_2O_3, Wt. %
1	SiO_2–Al_2O_3	4.54
		4.56
2	SiO_2–Al_2O_3	7.73
		7.69
3	SiO_2–Al_2O_3	9.20
		9.22
4	SiO_2–Al_2O_3	14.73
		14.77
5	SiO_2–Al_2O_3	17.70
		17.65
6	SiO_2–Al_2O_3	26.60
		26.64
7	SiO_2–Al_2O_3	30.60
		30.50

The precision that is obtained when the procedure is applied on a routine basis is illustrated by the results in Table IV. These analyses were made on a few typical catalyst samples that were submitted to the analytical laboratory for aluminum determinations.

DISCUSSION

The volumetric method reduces the time required for a determination of aluminum in catalysts from about 8 hours by the gravimetric method to about 2 hours. This means a saving not only in time but also in expensive platinum apparatus.

It is necessary that the hydrochloric acid be standardized against standard aluminum sulfate solutions. The normality of the solution used in the work reported here was about 3% higher when determined by the titration of aluminum than when standardized against sodium carbonate. Since this work was completed, it has been found that aluminum potassium sulfate can be used as a primary standard for the hydrochloric acid solution.

When fairly large amounts of other ions that form complexes with tartrate are present, more of the sodium tartrate solution must be added or low results will be obtained. Examples of such ions are lead, iron, and magnesium.

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Activated Glycerol Dichlorohydrin

A New Colorimetric Reagent for Vitamin A

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Activated glycerol dichlorohydrin is suggested as a new reagent to replace glycerol dichlorohydrin as obtained from various manufacturers, which has been found unreliable as a colorimetric reagent for vitamin A. The activated reagent is prepared by vacuum distillation of glycerol dichlorohydrin with antimony trichloride. A spectrophotometric study was made of the violet color produced by the addition of activated glycerol dichlorohydrin to a solution of vitamin A in chloroform. The color has been found to be stable from 2 to 10 minutes after addition of the reagent at the wave length of maximum absorption, 555 mμ. The color obeys Beer's law over a wide range. Free and esterified vitamin A give the same extinction coefficient with the reagent. The interference of vitamin D_2 and related sterols is negligible. Vitamin A and carotene can be determined simultaneously by reading at 555 and 800 mμ on the Beckman spectrophotometer or carotene interference can be evaluated by the method of Dann and Evelyn. The activated reagent is unaffected by traces of moisture found in the atmosphere on the most humid days, and leaves no deposit of antimony oxychloride on the cuvettes.

RECENTLY (*4*) the authors proposed the use of practical glycerol dichlorohydrin as a new reagent for the quantitative estimation of vitamin A. Later it was found that glycerol dichlorohydrin from other firms, and most of that from Eastman Kodak, did not give color when added to vitamin A.

By distilling glycerol dichlorohydrin with 1 to 5% antimony trichloride at 4- to 40-mm. pressure, a reagent was obtained which reacted with vitamin A as did the practical material. Activation could also be obtained by the addition of concentrated hydrochloric acid, acetyl chloride, phosphorus pentachloride, anhydrous aluminum chloride, and benzoyl chloride. Concentrated sulfuric acid, zinc chloride, and stannic chloride gave blue colors which did not change to violet. Glycerol 2,3-dichlorohydrin, glycerol 1,3-dibromohydrin, and glycerol 2,3-dichlorohydrin when activated gave blue colors changing to violet. Acetylated glycerol dichlorohydrin does not react with vitamin A even when activated.

The concentration of antimony calculated as the trichloride in the activated reagent varied from a trace to 0.67%. The activation of the reagent, however, is not due to antimony trichloride per se, since glycerol dichlorohydrin to which 0.1 to 1.0% of antimony trichloride is added is inactive toward vitamin A.

Although activated and practical glycerol dichlorohydrin react in an apparently similar manner with vitamin A, several differences were observed which indicates that they are not identical. (1) The absorption spectrum produced on the addition of carotene to the two reagents is different (see Figure 1). Between 400 and 550 mμ the color given with activated glycerol dichlorohydrin has a much lower absorption curve than the color produced with the practical reagent. (2) With the practical reagent at 550 mμ at the end of 15 minutes the L values of vitamin D_2, ergosterol, and 7-dehydrocholesterol are 11.28, 1.13, and 0.08 (*3*), respectively, while with the activated reagent the L values are 6.90, 0.25, and 0.29, respectively. [The term $L^{1\%}_{1\ cm.}$ was introduced by Dann and Evelyn (*1*) for use with photoelectric colorimeters which employ a band width of light 30 to 40 mμ wide. The authors have used the term with a Coleman universal spectrophotometer model 11 because it employs a band of light 35 mμ wide (*4*). Throughout this paper $L^{1\%}_{1\ cm.}$ is used for work done on the Coleman model 11 spectrophotometer, and $E^{1\%}_{1\ cm.}$ for work done on the Beckman quartz spectrophotometer. (3) The L value with vitamin A of the practical glycerol dichlorohydrin varied between 1010 and 1100, that of the activated reagent varied between 1100 and 1270.

A study was made of the reproducibility of the activated reagent under constant use and kept at room temperature. From Table I it can be seen that three different batches of glycerol dichlorohydrin vacuum-distilled with 2% antimony trichloride gave average $L^{1\%}_{1\ cm.}$, 550 mμ, at 2 minutes, using concentrations of vitamin A between 2 and 5 micrograms, of 1210.

Table I. Stability of Reagent Glycreol Dichlorohydrin Distilled with 2% Antimony Trichloride

Batch No.	Date Tested	$L^{1\%}_{1\ cm.}$, λ 550 mμ: Average value for 2 to 5 γ of vitamin A	$L^{1\%}_{1\ cm.}$, λ 550 mμ: Single determination on 2 to 5 γ of vitamin A
1	June 6	1210 ± 33	...
	June 14	.....	1210
	June 20	.....	1270
	June 27	.....	1240
	July 9	.....	1200
	July 16	.....	1260
2	Aug. 7	1230 ± 42	...
	Aug. 29	.....	1230
	Sept. 7	.....	1190
3	Sept. 24	1170 ± 17	...
	Oct. 2	.....	1165
	Oct. 8	1140 ± 4	...
	Oct. 15	1160 ± 14	...
	Oct. 22	.....	1180
	Oct. 26	.....	1160
	Nov. 2	.....	1140
	Dec. 17	.....	1115

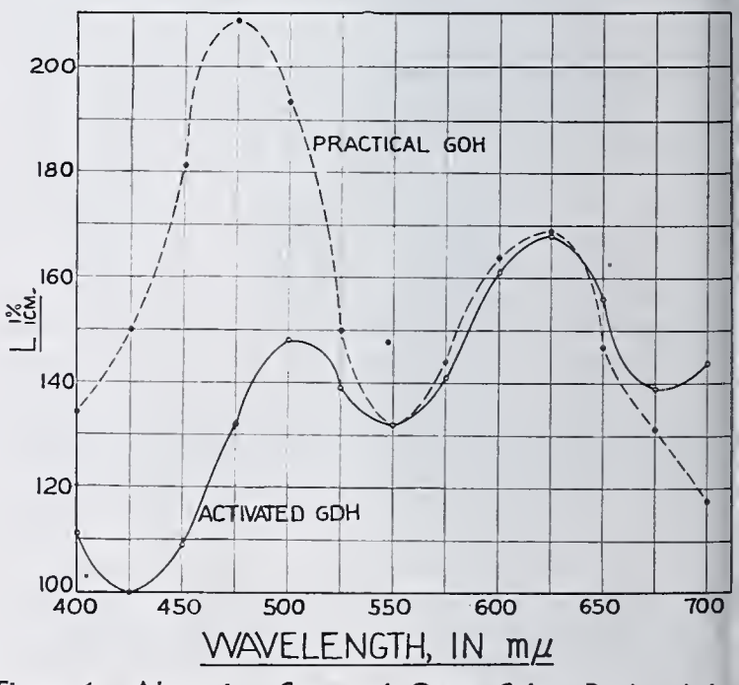

Figure 1. Absorption Curves of Green Colors Produced by Activated and Practical Glycerol Dichlorohydrin on Carotene

90% of β, 10% of α. 5.0 ml. of reaction mixture contained 108.8 micrograms of carotene. Readings taken on Coleman spectrophotometer

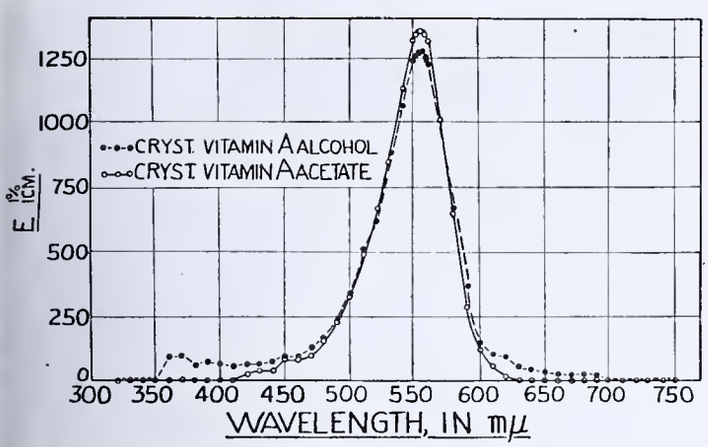

Figure 2. Absorption Curves of Violet Colors Produced by 4.89 Micrograms of Alcohol and 4.90 Micrograms of Acetate with Activated Glycerol Dichlorohydrin

Total volume of reaction mixture in each case, 5.0 ml. Readings taken on Beckman spectrophotometer

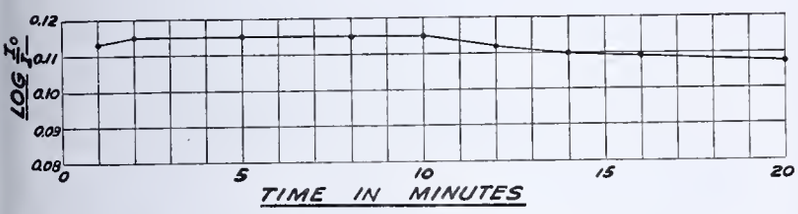

Figure 3. Stability of Violet Color Produced by Action of 4.07 Micrograms of Vitamin A with Activated Glycerol Dichlorohydrin

As shown by density readings on Beckman spectrophotometer at 555 mμ. Total volume of reaction mixture, 5.0 ml.

1235, and 1170. Although there is a 10% difference in the $L^{1\%}_{1\text{ cm.}}$ values between batches 1 and 3, it has been the authors' experience that when reagents are prepared from the same batch under constant conditions of pressure and antimony trichloride concentration, reproducible reagents result. (By reproducible reagents are meant those that give the same $L^{1\%}_{1\text{ cm.}}$ with vitamin A.) One batch of activated glycerol dichlorohydrin kept in a Pyrex bottle in the absence of light lost only 5% of its activity in 14 months.

From Table I can be had a measure of the stability of the reagent. The third column gives the average $L^{1\%}_{1\text{ cm.}}$ value and the average deviation from this value for three batches of activated glycerol dichlorohydrin, for concentrations of vitamin A between 2 and 5 micrograms. In the last column are presented $L^{1\%}_{1\text{ cm.}}$ values for single determinations for one particular concentration of vitamin A between 2 and 5 micrograms. It can be seen from the table that the reagent possesses good stability for about 2 months. After that time there is a slow decrease in the $L^{1\%}_{1\text{ cm.}}$ value given by the reagent.

In Figure 2 is presented the absorption spectrum taken on the Beckman spectrophotometer from 320 to 750 mμ of the violet color produced by the reaction of activated glycerol dichlorohydrin with crystalline vitamin A alcohol and crystalline vitamin A acetate. In order to be within the period of stability for the maxima, the readings were taken from 2 to 10 minutes after mixing the reagents because the violet color was found to be stable from 2 to 10 minutes, as seen in Figure 3.

The $E^{1\%}_{1\text{ cm.}}$ of the color produced by the action of activated glycerol dichlorohydrin on the crystalline alcohol was 1270 at its maximum wave lengths of 553 to 556 mμ, while the $E^{1\%}_{1\text{ cm.}}$ of the color produced with the crystalline acetate at its maximum wave lengths of 553 to 556 mμ was 1350 calculated for vitamin A content.

The shapes of the two curves are very similar. The smaller extinction of the alcohol compared to that of the acetate may have been due to either of two reasons: deterioration of the alcohol or an inherent difference in the reaction of the alcohol and acetate with activated glycerol dichlorohydrin.

In order to determine which of these conclusions was the correct one, the extinctions of the colors produced by the action of activated glycerol dichlorohydrin on a distilled natural ester concentrate and upon the unsaponifiable fraction of the same concentrate were calculated from the observed density readings at the maxima. Figure 4 shows the absorption curves of these colors. For the whole oil, the $E^{1\%}_{1\text{ cm.}}$ at the maxima of 553 to 556 mμ was 1420, while that for the unsaponifiable extract was 1410. Obviously, there is no appreciable difference in the behavior of the alcohol and ester toward glycerol dichlorohydrin, between 400 to 750 mμ, and the lower extinction observed above is probably due to some deterioration of the crystalline alcohol. (The $E^{1\%}_{1\text{ cm.}}$ 325 mμ, in absolute ethanol of the crystalline vitamin A alcohol was 1740, and its extinction ratios of 300/328 and 350/328 mμ were 0.69 and 0.49, respectively.) The upward trend of the curve from 400 to 320 mμ was due to something in the particular batch of concentrate used which reacted with the activated reagent, because using the same batch of reagent and other batches of concentrate, the upward sweep was not observed, nor was it observed with crystalline vitamin A alcohol and acetate (see Figure 2).

Since most of the authors' work on vitamin A determinations is done on the less expensive Coleman model 11 spectrophotometer, the absorption spectrum of the violet color produced with the whole oil of the above concentrate and activated glycerol dichlorohydrin was read from 400 to 700 mμ on this instrument. It is plotted in Figure 5 along with the absorption spectrum of the same color taken on the Beckman spectrophotometer. On the Coleman, the $L^{1\%}_{1\text{ cm.}}$ was 1140 at the maximum of 550 mμ, while on the Beckman the $E^{1\%}_{1\text{ cm.}}$ was 1420 at the maximum wave lengths of 553 to 556 mμ. Not only has the wave length of the maximum shifted in using the Coleman, but the height of the maximum has decreased and is more flat. This is to be expected, since the Coleman employs a band width of about 35 mμ as compared to the band width of 5 mμ or less which the authors used with the Beckman instrument. Similar results were obtained by Mellon (*2*) using two spectrophotometers, one with a band width of 5 mμ, the other with a band width of 35 mμ. Upon plotting the per cent transmittancy of a Corning glass 512, he found that with

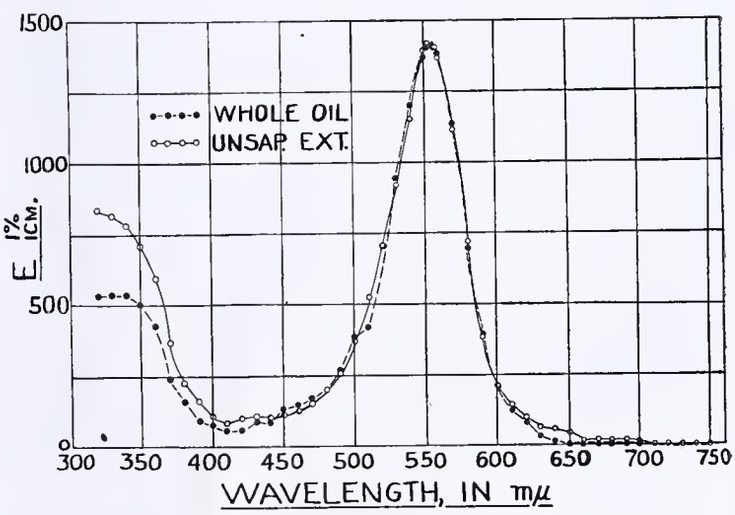

Figure 4. Absorption Curves of Colors Produced by Activated Glycerol Dichlorohydrin on a Natural Vitamin A Ester Concentrate (3.71 Micrograms of Vitamin A) and on Unsaponifiable Fraction of Same Batch of Concentrate (4.47 Micrograms of Vitamin A)

Total volume of reaction mixture in each case, 5.0 ml. Readings taken on Beckman spectrophotometer

Table II. Relation between Concentration and Extinction Coefficient of Color Produced on Addition of Activated Glycerol Dichlorohydrin to Vitamin A

Concentration of Vitamin A, γ/5 Ml.	$E^{1\%}_{1\text{ cm.}}$ 555 mμ[a]	$L^{1\%}_{1\text{ cm.}}$ 550 mμ[b]
0.94	1020	1060
1.36	1250	1130
1.87	1290	1140
2.73	1340	1140
2.81	1300	1110
3.74	1330	1110
4.68	1330	1100
6.82	1310	1060
8.18	1290	1050
9.36	1290	1010
13.63	1250	970
18.71	1180	900
28.07	1090	800

[a] Beckman spectrophotometer.
[b] Coleman.

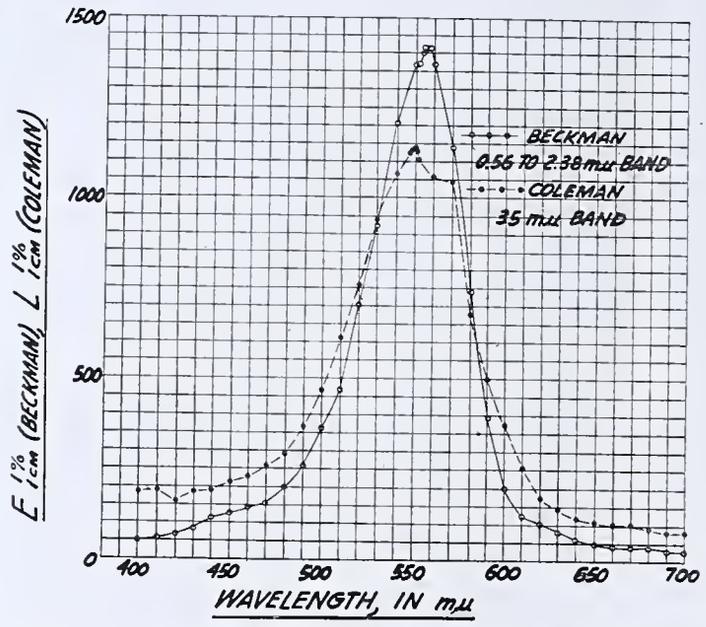

Figure 5. Absorption Spectrum on Beckman and Coleman Spectrophotometers of Violet Color Produced by Activated Glycerol Dichlorohydrin with 4.82 Micrograms of Vitamin A of a Whole Vitamin A Natural Ester Concentrate

Total volume of reaction mixture in each case, 5.0 ml.

the latter type of instrument some maxima not only were decreased (had lower extinction values) and shifted, but might even be eliminated altogether.

Figure 6 shows the density per unit of absorption path as a function of the concentration of vitamin A from 1 to 28 micrograms for both the Coleman and Beckman spectrophotometers. Density and per cent transmission readings were made at the end of 2 minutes. Table II shows the $E^{1\%}_{1\text{ cm.}}$ and $L^{1\%}_{1\text{ cm.}}$ for various concentrations of vitamin A. On the Beckman, a nearly linear relation was observed between 1.36 and 13.63 micrograms of vitamin A. Between these two values, the average extinction was 1300, with an average deviation of ±24. On the Coleman a nearly linear relation was observed within a smaller range of vitamin A concentration than on the Beckman. Between 1.36 and 4.68 micrograms, the average $L^{1\%}_{1\text{ cm.}}$ was 1120 ± 15. Although higher concentrations of vitamin A were not tested with this batch of reagent, the authors have found that the nearly linear relation does hold up to about 5.5 micrograms on the Coleman.

The interference of carotene, vitamin D_2, and other related sterols in the estimation of vitamin A with activated glycerol dichlorohydrin was studied by measuring the absorption of the colored solutions produced under the same conditions as were employed for vitamin A measurements. As seen in Table III, the interferences with the exception of carotene are negligible. The error due to an equal weight of carotene is 11.0% when the Coleman spectrophotometer is used and 7.35 with the Beckman spectrophotometer. This may be compared with a 5% interference in the antimony trichloride method.

The carotene interference may be evaluated by the method of Dann and Evelyn (*1*) with activated glycerol dichlorohydrin, just as in the case of antimony trichloride.

A second method of evaluating carotene is suggested from the data plotted in Figure 7, where the absorption spectra of crystalline vitamin A acetate and carotene treated with activated glycerol dichlorohydrin are compared. Above 630 mμ the vitamin A color no longer absorbs light, whereas the carotene color continues absorbing light beyond this point. Thus, by taking readings at two wave lengths, 555 and 800 mμ, one can evaluate both carotene and vitamin A, as the optical densities are additive as shown in Table IV.

APPARATUS

A Beckman Model DU Quartz Spectrophotometer with a tungsten light source and Corex cells of 1.00-cm. absorption path was employed for the region of 320 to 750 mμ. The wavelength setting of the instrument was calibrated with 405 and 546 mμ lines obtained by using a mercury arc as a light source. Throughout the measurements the sensitivity knob was kept at three turns from its clockwise limit. The slit width was varied to get 100% transmission of the blank. The following table shows the variation of slit width and band width with the wave length for readings of the violet color produced with activated glycerol dichlorohydrin.

Wave Length Range, mμ	Slit Width Variation, Mm.	Band Width Variation, mμ
320–390	1.8–0.15	8.16–1.41
400–500	0.075–0.028	0.75–0.56
500–600	0.028–0.042	0.56–1.43
600–630	0.042–0.064	1.43–2.38
630–700	0.064–0.040	2.38–1.90

Readings were taken at 1 mμ intervals near the maxima, otherwise at 10 mμ intervals.

Coleman Universal Spectrophotometer, model 11, was used for measurements with a PC-4 filter in the region of 400 to 700 mμ. The instrument was calibrated according to the directions given by the manufacturer. This instrument employs a band of light 35 mμ wide (which can be located with a precision of less than 2 mμ). For this reason $L^{1\%}_{1\text{ cm.}}$ values are reported instead of $E^{1\%}_{1\text{ cm.}}$ values, which are used for monochromatic light. Direct readings of per cent transmission were made on the galvanometer scale by setting the blank at 100. The absorption path was 1.3 cm. Readings were taken at 10 mμ intervals.

REAGENTS

Glycerol Dichlorohydrin, practical grade of Eastman Kodak. 1,3-dichloro-2-hydroxypropane from Ohio Chemical Mfg. Co. A mixture of the 1,3- and 2,3-dichlorohydrins from the Shell Chemical Co.

Activated Glycerol Dichlorohydrin. To 1000 ml. of any of the above glycerol dichlorohydrins were added 100 ml. of chloroform containing 10 to 50 grams of dissolved antimony

Table III. Interference of Carotene, Vitamin D_2, and Related Sterols on Reaction of Glycerol Dichlorohydrin with Vitamin A

		Concentration	Time after Adding Reagent· 2 Min.	5 Min.	10 Min.	Interference at 2 Minutes
		Mg./100 ml.				%
$E^{1\%}_{1\text{ cm.}}$ λ 555	Vitamin A	0.06	1390	1390	1390	
$E^{1\%}_{1\text{ cm.}}$ λ 555	Carotene	1.31	102	114	122	7.35
$L^{1\%}_{1\text{ cm.}}$ λ 550	Vitamin A	0.06	1150	1150	1150	
$L^{1\%}_{1\text{ cm.}}$ λ 550	Carotene	2.18	126	132	132	11.00
$L^{1\%}_{1\text{ cm.}}$ λ 550	Calciferol	62.6	1.79	4.03	6.25	0.16
$L^{1\%}_{1\text{ cm.}}$ λ 550	Ergosterol	52.2	0.14	0.14	0.19	0.01
$L^{1\%}_{1\text{ cm.}}$ λ 550	7-Dehydrocholesterol	33.4	0.21	0.21	0.21	0.02
$L^{1\%}_{1\text{ cm.}}$ λ 550	Cholesterol	160.0	0.00	0.00	0.00	0.00

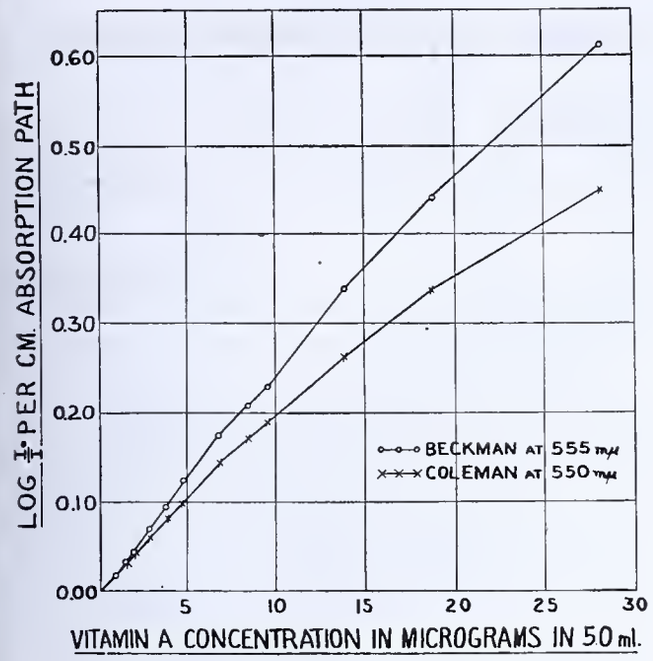

Figure 6. Relation between Absorption at Maximum, and Vitamin Concentration

Total volume of reaction mixture, 5.0 ml.

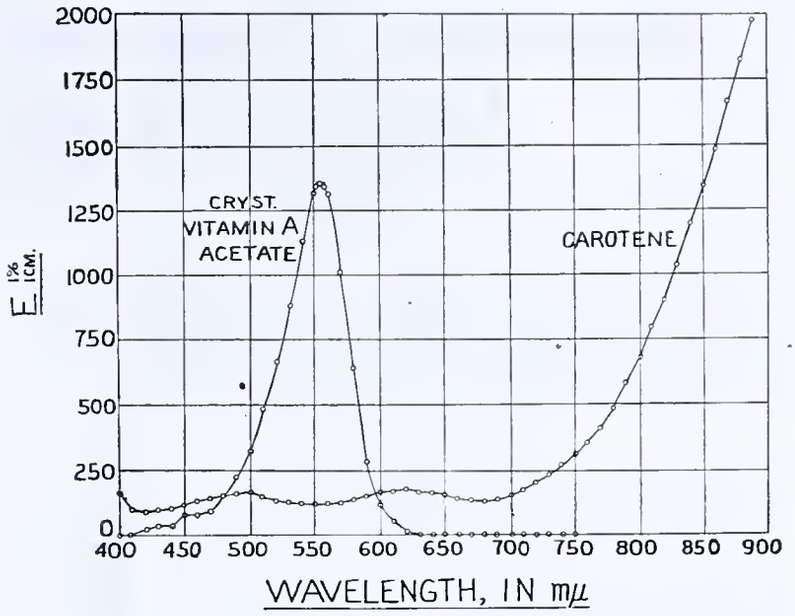

Figure 7. Absorption Curves of the Colors Produced by Activated Glycerol Dichlorohydrin on 4.90 Micrograms of Vitamin A Acetate and 65.44 Micrograms of Carotene

90% of β, 10% of α. Total volume of the reaction mixture in each case, 5.0 ml. Readings taken on Beckman spectrophotometer

trichloride (prepared without anhydrous precautions). Later, it was found that the solid can be added directly. The solutions were mixed and distilled under a reduced pressure of 4 to 40 mm. of mercury in an all-glass apparatus. The chloroform fraction was discarded and the product collected and stored in a dry glass-stoppered bottle at room temperature. The dichlorohydrins from the Shell Chemical Co. were somewhat less satisfactory in the preparation of activated glycerol dichlorohydrin because they required two distillations with antimony trichloride before a colorless reagent was obtained. The product thus obtained became colored in 3 to 4 weeks.

Chloroform, analytical reagent grade, dried over anhydrous sodium sulfate, distilled, and kept over the same drying agent.

Antimony Trichloride, reagent grade, Merck.

Crystalline Vitamin A Alcohol and crystalline vitamin A acetate from Distillation Products, Inc.

Distilled Vitamin A Natural Ester Concentrate, Control No. PC-3, from Distillation Products, Inc., which comes in sealed gelatin capsules. The $E^{1\%}_{1\,cm.}$ of the concentrate in absolute ethanol was 100.75. Taking the $E^{1\%}_{1\,cm.}$ from crystalline vitamin A as 1780, the concentrate was found to contain 5.65% vitamin A. The concentrate from each capsule was used for one day only. The above data for the oil were supplied by Distillation Products, Inc., and were confirmed by the authors' measurements.

COLOR DEVELOPMENT

All dilutions were made, and all colors were developed in a constant-temperature bath at 25° C.

To 4.0 ml. of activated glycerol dichlorohydrin in a 10-ml. glass-stoppered cylinder, was added 1 ml. of chloroform containing the desired concentration of vitamin A, carotene, or both. The mixture was shaken several times to ensure homogeneity and placed in the water bath until ready to be read in the spectrophotometer.

All density readings reported in this paper are the averages of duplicate determinations.

SUMMARY

Glycerol dichlorohydrin from various sources does not always produce color with vitamin A. By distilling glycerol dichlorohydrin with 1 to 5% of antimony trichloride under reduced pressure, a reagent results which behaves similarly to practical glycerol dichlorohydrin in giving a transient blue, then violet, color with vitamin A which is stable for 8 minutes, is not affected by traces of moisture present in the atmosphere on the most humid days, and does not leave a white deposit of antimony oxychloride on the cuvettes. The activated reagent differs from the practical in its behavior toward carotene and various sterols.

Table IV. Mixed Color Method for Estimation of Vitamin A and Carotene Mixtures by Activated Glycerol Dichlorohydrin

Vitamin A	Carotene	Observed Density		Calculated Density	
		555 mμ	800 mμ	555 mμ	800 mμ
γ/ml.	γ/ml.				
3.98	...	0.115	0.00	...	...
...	10.88	0.027	0.136	...	...
...	21.76	0.056	0.291	...	...
...	32.64	0.085	0.439	...	...
...	43.52	0.113	0.580	...	...
3.98	10.88	0.146	0.131	0.142	0.136
3.98	21.76	0.173	0.284	0.171	0.291
3.98	32.64	0.198	0.442	0.200	0.439
3.98	43.52	0.224	0.580	0.228	0.580

The absorption spectrum was taken on the Beckman spectrophotometer between 320 and 750 mμ of the violet color produced by the action of activated glycerol dichlorohydrin with crystalline vitamin A alcohol, crystalline vitamin A acetate, a natural ester concentrate, and its unsaponifiable fraction. In each case the observed maxima were between 553 and 556 mμ. The $E^{1\%}_{1\,cm.}$ of the vitamin A in the natural ester concentrate and its unsaponifiable fraction were almost identical, 1420 and 1410, respectively. A nearly linear relationship was observed at 555 mμ between 1.26 and 13.63 micrograms of vitamin A and optical density.

On the Coleman model 11 spectrophotometer, the maximum has shifted to 550 mμ. The $L^{1\%}_{1\,cm.}$ value is about 20% lower than the $E^{1\%}_{1\,cm.}$ value on the Beckman. On the Coleman, a nearly linear relationship between vitamin concentration and optical density existed between 2 and 5.5 micrograms of vitamin A.

Under the conditions employed for vitamin A determinations using activated glycerol dichlorohydrin, the interference of vitamin D and related sterols is negligible.

The carotene interference can be evaluated by the method of Dann and Evelyn or by reading at 555 and 800 mμ on the Beckman spectrophotometer, since the violet vitamin A color does not absorb at 800 mμ.

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Amperometric Titration of Cyanide with Silver Nitrate, Using the Rotating Platinum Electrode

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The amperometric titration of cyanide with silver is equal in accuracy and precision to the visual Denigès method, and is applicable at much higher dilution. Chloride, bromide, hydroxide, and high concentrations of potassium nitrate or sulfate do not interfere.

IT HAS been shown by Thompson (*19*), who used very pure potassium cyanide as a primary standard substance, that the silver nitrate titration of cyanide to $Ag(CN)_2^-$ is more accurate than the mercuric chloride titration Wick (*21*) showed, however, that the potentiometric mercuric chloride titration is accurate. The original method of Liebig (*12*), based on the formation of a turbidity due to silver cyanide, is subject to error in alkaline (*3*, *18*) and ammoniacal (*8*, *21*) solution. The Denigès (*4*) titration, based on a turbidity due to silver iodide in the presence of ammonia, gives high results in the presence of a large excess of ammonia (*8*, *9*, *20*, *21*), but yields accurate results if the concentration of ammonia is carefully regulated (*8*, *9*, *19*, *21*).

The potentiometric titration of cyanide with silver has been described by Treadwell (*20*), Müller and Lauterbach (*16*), and Clark (*2*). Wick (*21*) concluded that the potentiometric titration gives accurate results. Read and Read (*17*) suggested a bimetallic electrode titration, and Gregory and Hughan (*6*), using the null-point equivalence potential method of Cavanagh (*1*), report that the potentiometric method is superior to the Liebig method (*5*) for the determination of cyanide in plating solutions. The potentiometric method has also been used for the indirect determination of nickel (*14*), cobalt (*15*), and zinc (*7*, *13*).

The successful amperometric titrations of halides with silver nitrate using the rotating platinum electrode (*10*, *11*) suggested a similar method for cyanide. The present paper describes an accurate comparison of the amperometric end point with the Denigès titration, which has been proved to be accurate to within 0.2% and probably 0.1% by reference to pure potassium cyanide (*19*) and to the potentiometric end point (*19*, *21*).

EXPERIMENTAL

A 0.500 *N* solution of reagent quality silver nitrate and a 1.0 *M* solution of C.P. potassium cyanide in 0.1 *N* sodium hydroxide were compared accurately, using weight burets, finishing each titration with 0.01 *N* silver nitrate prepared by diluting a known weight of stock solution to a known volume, and using a volume buret for the final titration. The end point was observed by both the visual (Denigès) and amperometric methods. No blank correction was applied to the visual end point.

For the amperometric titration, the apparatus and technique previously described (*10*) were used, with the mercury–mercuric iodide–potassium iodide reference electrode and a galvanometer sensitivity of about 0.02 microampere per mm. The residual current before the end point was extremely small, and very sharp end points were observed. Between titrations, the silver was removed from the platinum electrode by anodic polarization or by using nitric acid to prevent an anodic residual current due to dissolution of silver in the cyanide solution. If nitric acid was used to remove the silver, the electrode was allowed to stand in ammonium hydroxide solution for a few minutes before rinsing with water and using it again.

In Table I, experimental data are presented for the titration of 20-gram samples of 1.0 *M* potassium cyanide, diluted to about 100 ml. with 0.1 *N* sodium hydroxide to give a final cyanide concentration of 0.2 *M* (0.1 *N* with respect to silver). For purposes of calculation, the silver nitrate solution was assumed to be exactly 0.5 *N* (weight basis). The two end points are comparable in precision and yield the same answer to about 0.1% in the titration of 0.1 *M* cyanide.

To compare the two end points for more dilute solutions of cyanide, accurate dilutions of the two stock solutions were made by diluting known weights of stock solution to known volumes. All dilutions were carried out with 0.1 *N* sodium hydroxide, to prevent loss of hydrogen cyanide by hydrolysis. The dilute solutions were then compared by the usual volumetric technique using as a basis of calculation the average normality of stock cyanide solution determined by the visual end point (Table I). The results of the comparison are given in Table II.

The two end points were comparable in precision and accuracy at cyanide concentrations as low as 0.002 *N*.

The visual end point became indistinct at 2×10^{-4} *N* cyanide and failed at higher dilutions, whereas a distinct amperometric end point was still observed in titrating 4×10^{-6} *N* cyanide (8×10^{-6} *M*) with 5×10^{-4} *N* silver nitrate. In general, it is recommended that for the amperometric titration a fivefold more concentrated silver solution than cyanide be used to avoid the necessity of correcting the current for dilution effect. For the titration of very dilute cyanide, the lowest practical limit for silver nitrate concentration is of the order of 5×10^{-4} *N* to obtain a distinct end point.

Table I. Comparison of Visual and Amperometric End Points in Titration of 0.1 *M* Cyanide

V = visual, A = amperometric end point

KCN Solution	$AgNO_3$ Solution		Normality of Stock KCN, Calcd.	Deviation from Mean	End Point
Grams	*Grams*			%	
19.737	20.068		0.50839	−0.07	V
19.806	20.168		0.50914	+0.08	V
19.142	19.479		0.50880	+0.01	V
20.470	20.828		0.50874	0.00	V
20.185	20.532		0.50864	−0.03	V
		Av.	0.50873	±0.04	
19.853	20.173		0.50808	+0.02	A
18.763	19.058		0.50786	−0.06	A
20.920	21.278		0.50856	+0.08	A
19.967	20.302		0.50839	+0.05	A
19.911	20.225		0.50791	−0.05	A
		Av.	0.50816	±0.05	

Table II. Comparison of Visual and Amperometric Titration of 100 Ml. of Dilute Potassium Cyanide in 0.1 *N* Sodium Hydroxide with Silver Nitrate

Approximate Normality of Cyanide	End Point	% Error	Average Error, %
0.02	Visual	+0.3, +0.2, +0.3, +0.5, +0.1	+0.3
	Amperometric	−0.3, −0.3, −0.4, −0.4, −0.4	0.4
$\times 10^{-3}$	Visual	+1.0, +0.8, +0.8, +0.7, +0.7	+0.8
	Amperometric	−0.4, −0.1, −0.1, −0.1, −0.1	−0.2
2×10^{-4}	Visual	−0.3, −3.1, −3.3, −3.3, −3.6	−2.7
	Amperometric	−2.7, −2.7, −2.6, −2.4	−2.6
2×10^{-5}	Visual	No end point	
	Amperometric	+6.5, −1.6, +1.1, +0.7, +4.9	+2.3
4×10^{-6}	Visual	No end point	
	Amperometric	+9.2, +12.8, +2.3, +2.3, −2.3	+4.9

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able III. Effect of Salts and Hydroxide in Titration of 0.002 *N* Cyanide

(In 0.1 *N* sodium hydroxide unless otherwise stated)

Salt Added	% Error	Average Error, %
0.001 *N* KCl	+0.6, +0.6, +0.4	+0.5
0.004 *N* KCl	+0.4, +0.3, +0.1	+0.3
0.1 *N* KCl	+0.4, −0.2, +0.2	+0.1
0.001 *N* KBr	−0.1, +0.6, +0.5	+0.3
0.004 *N* KBr	+0.3, 0.0, +0.7	+0.3
0.1 *N* KBr	+0.4, +0.4, +0.4	+0.4
1.0 *N* KNO_3 + 0.02 *N* NaOH	−0.2, +0.1, −0.4	−0.2
1.0 *N* KNO_3 + 1.0 *N* NaOH	−0.8, −0.8, −0.6	−0.7
0.5 *M* K_2SO_4 + 0.02 *N* NaOH	−0.2, +0.1, −0.1	−0.1
Satd. K_2SO_4 + 1.0 *N* NaOH	−0.8, −0.6, −0.2	−0.5

EFFECT OF CHLORIDE, BROMIDE, HYDROXIDE, AND HIGH CONCENTRATIONS OF SALTS

A series of titrations of 0.002 *N* cyanide in the presence of arious concentrations of chloride, bromide, hydroxide, nitrate, nd sulfate was carried out (Table III). The errors again were omputed with reference to the visual end point titration of 0.1 *N* yanide. In general the results are accurate to within 0.5% in the resence of 50-fold excess of chloride or bromide or in 1 *N* salt olutions. High concentrations of alkali tend to give low results.

INTERFERENCES

Iodide, sulfide, sulfhydryl compounds, etc., which form extremely insoluble silver salts, or materials which form more table complexes with silver than cyanide does, will, in general, nterfere.

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Vapor-Liquid Equilibrium Still for Miscible Liquids

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A new apparatus is described for the determination of vapor-liquid equilibrium data; it consists of an electrically heated still fitted with a Cottrell pump, a vapor-liquid disengagement chamber, and a condensate trap. Both the boiling liquid and the vapor circulate within the apparatus, and boiling points may be determined with accuracy, as the system ensures complete equilibrium between the two phases. A simple test for the entrainment of liquid in the vapor is described, and it is shown that in the new apparatus less than 0.05% entrainment occurs with negligible effect upon equilibrium data.

VAPOR-liquid equilibrium determinations reported by different investigators frequently show wide inconsistencies, and it is only rarely that a new examination of a system confirms previous results. Very few systems are known to form ideal solutions, whereby the vapor-liquid relationships may be calculated by Raoult's law from vapor pressures, and the engineer must usually depend upon experimental data for the design of distillation and other contacting equipment. It is, therefore, not surprising to find that such equipment frequently does not operate to the predicted specifications, and the need for greater reliability in equilibrium determinations is very evident. There is also a need for the accurate measurement of other physical properties of systems, such as boiling points and vapor pressure–temperature relationships, which enable the calculation of activity coefficients for the mathematical correlation and extension of results.

Many different forms of apparatus have been proposed for the examination of the vapor-liquid relationships of miscible liquids. Some of the more recent are those of Jones, Schoenborn, and Colburn (*5*), Langdon and Keyes (*6*), Othmer (*7*), and the modifications by York and Holmes (*10*). From a careful analysis of these and other forms, it would appear that no one type of apparatus so far described is entirely free from possible sources of error, and a still operating on a new principle is described in this paper in the hope that it may assist in progress towards the elimination of these faults, and at the same time make it possible to determine accurately the true boiling points of the mixtures being investigated.

Various methods have been adopted to prevent partial condensation and refluxing of the vapors. These methods fall generally into one or other of two main classes, in which either the vapor line is jacketed with the same vapors, or some form of external heating is applied to the exposed vapor-conveying sections, respectively. Combinations of the two methods are also described. Vapor-jacketing when used alone does not appear to be a fully effective means of reflux-prevention. This is particularly evident with liquids of high boiling point, with which refluxing from the jacketed vapor line may become visibly quite pronounced. Further, in considerations of apparatus of this type (*7*), the enrichment of the vapors with respect to the more volatile component before they reach the vapor line does not appear always to have been recognized. It is obvious that this enrichment can occur by partial condensation on the outer body of the still at all points above the level of the boiling liquid.

In several forms of apparatus, external heating is adopted to compensate for heat losses from the upper parts of the still (*1*, *5*, *6*, *8*, *10*) and so prevent refluxing of the vapors, although the danger of evaporation of thin films and spray droplets on such heated surfaces has been pointed out (*7*). Despite this possibility of error, the method appears to have been used successfully. Carey and Lewis (*1*) have surrounded the entire vaporization chamber with an accurately heated jacket, and the careful technique of these investigators has led Jones *et al.* (*5*) to concede that the possible sources of error in this type of apparatus may not be important when special precautions are taken. Nevertheless, it

must be considered a disadvantage that the accuracy of results should depend upon strict operating conditions which cannot always be defined clearly.

Very little attention has been given to the difficulties which are undoubtedly associated with ensuring equilibrium between a boiling liquid mixture and its vapor, and it has usually been assumed that the vapor attains this equilibrium during a relatively short passage through the liquid in the boiling vessel, or in a separate equilibrium chamber. This assumption has not been confirmed in ebullioscopic investigations, where, until the development of the Cottrell device (*4*), elaborate precautions and a skillful technique were necessary to obtain complete equilibrium, and hence a steady boiling point. It is difficult to assess the significance of this in vapor-liquid equilibrium determinations, but it is unlikely that the vapors produced by normal boiling in a flask are in true equilibrium with the main bulk of the liquid.

Of more obvious importance is the possibility of local heterogeneity within the boiling liquid, due to the returning condensed vapor. It has been assumed that the agitation induced by the boiling liquid is sufficient to ensure prompt and thorough mixing of the still contents (*7*). The validity of this assumption has been questioned by Langdon and Keyes (*6*), who found it necessary to provide mechanical agitation in their equilibrium still. Jones, Schoenborn, and Colburn (*5*) also point out that the returning liquid may tend to flash-vaporize because of its lower boiling point, but it is doubtful whether these authors achieve any improvement in this regard by returning the condensate as a somewhat superheated vapor. The heterogeneity of the still contents may be further aggravated by the fluctuations in the condensate level in the wide-bore return line usually adopted.

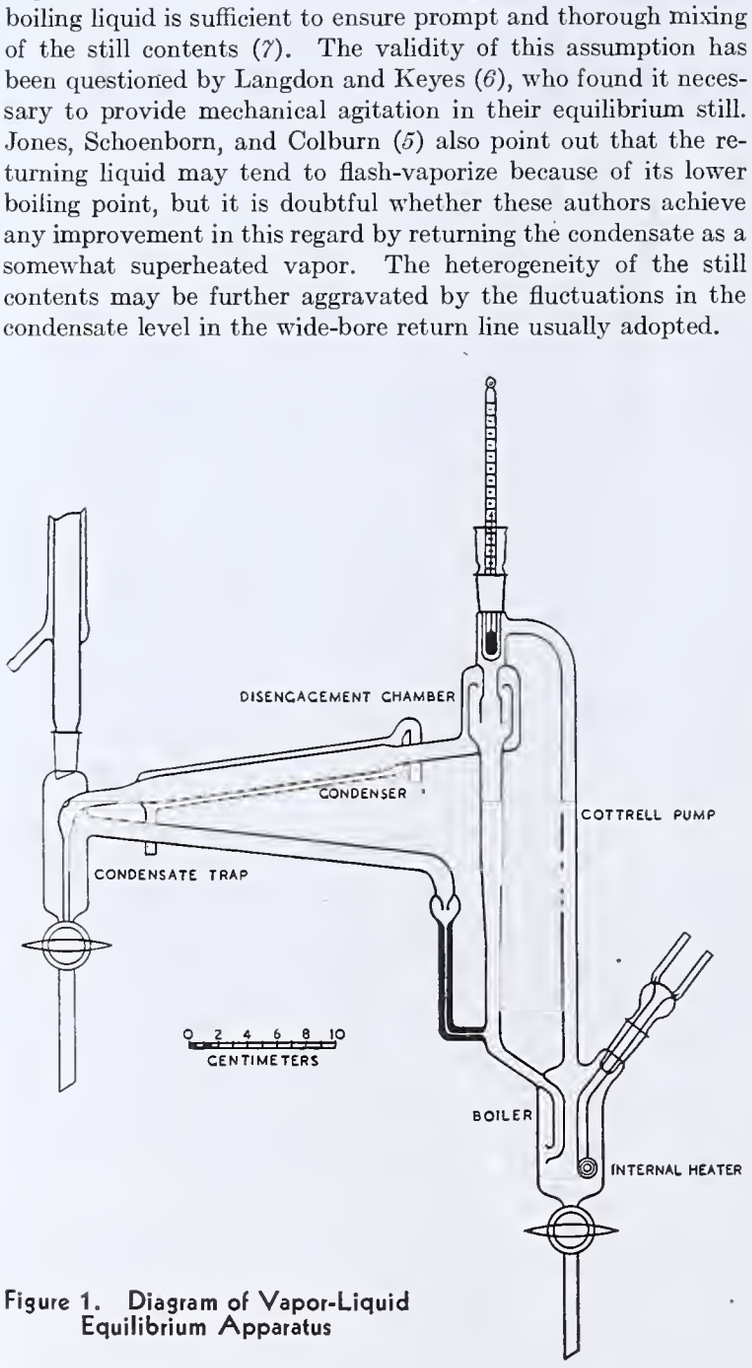

Figure 1. Diagram of Vapor-Liquid Equilibrium Apparatus

In work previously reported in the literature, it is usual for the steady operation of the equilibrium still to be interrupted in some way before taking samples of the boiling liquid and condensate. Errors may then be introduced into equilibrium data by changes in the composition of the still contents prior to and during sampling (*6*, *7*), either by loss of vapor from the still, or by the return of excess condensate during the period usually allowed for boiling to cease. Langdon and Keyes (*6*) were able to prevent the drainage of the condensate line in their apparatus, but found it necessary to analyze successive samples of the boiling liquid and extrapolate the data to obtain a value for the composition of the liquid at the commencement of the sampling period. More reliable results should be obtained if both liquid and condensed vapor samples are taken simultaneously, under such conditions that the equilibrium established within the apparatus is not disturbed.

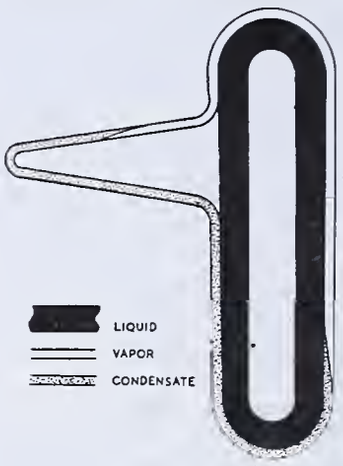

Figure 2

The need for accurate temperature data as well as vapor-liquid equilibrium compositions has been emphasized recently (*2*), but hitherto had received little attention. Precise temperature measurements may be ensured by the use of the Cottrell pump, which is incorporated in the equilibrium still due to Colburn, and described by Chilton (*3*) and Scatchard, Raymond, and Gilmann (*8*). Most forms of apparatus, however, provide for measurement of temperature in the vapor phase only, or in the boiling liquid in close proximity to the source of heat. If it could be assumed that under these conditions both the liquid and the vapor in contact with the thermometer represent the desired equilibrium conditions, either of these methods would be accurate, but it is usual for such data to show obvious inconsistencies in temperature measurements.

THE APPARATUS

In the design of the new apparatus, an attempt has been made to contribute further towards the elimination of the defects discussed above. The apparatus is illustrated in Figure 1. It is based on the well-established vapor-recirculation principle, but differs essentially from others of this type in that the boiling liquid is also circulated rapidly, as represented schematically in Figure 2, and in this regard the apparatus resembles that described by Swietoslawski (*9*) for the determination of boiling points. The liquid and vapor are not permitted to separate within the boiler, but are maintained in intimate contact as they pass up the modified Cottrell pump to the thermometer, and thence to the vapor-liquid disengagement chamber (see Figure 1). The conditions prevailing within the Cottrell tube during the operation of the apparatus are ideally designed to achieve the desired equilibrium between liquid and vapor and enable the boiling points of the mixtures under examination to be determined with complete accuracy. In the disengagement chamber the vapors are separated from the boiling liquid, and pass to the condensate system in such a manner that refluxing is virtually impossible. The stream of hot liquid disengaged from the vapor within the chamber is combined with the recycling condensate before it returns to the boiler, and it is clear that, at equilibrium the liquid-vapor mixture leaving the boiler is identical in quantity and composition with the combined liquid streams returning to it. This procedure is very convenient, as it ensures steadiness of composition within the boiler, and leaves no possibility of uneven flash-vaporization due to local points of heterogeneity.

The apparatus is sturdy, and may be readily constructed in Pyrex by glass-blowers of average ability. Variations in the dimensions may be made to suit the conditions of an investigation, but those indicated by Figure 1 will be found convenient for most systems, with possibly the exception of the wide Cottrell tube (10 mm. in outside diameter), which is designed primarily for the

v vapor density of systems der reduced pressure, and omewhat smaller tube (8 m. in outside diameter) ay be found better for essures around atmos- eric.

The relatively small boiler apacity approximately 0 cc.) is provided with th external and internal ating elements. The bulk the heat requirements ould be applied exter- lly, but the internal ele- ent will usually be found sential in order to main- in the steady boiling rate quired for the successful eration of the apparatus.

The external heater is ade by winding a suitable ngth of Nichrome wire a thin layer of as- stos paper, or micanite, ound the boiler, and subsequently covering with asbestos cord tape. The internal element may conveniently be made from .5 cm. (15 inches) of 28-gage platinum wire, compactly wound three concentric coils, and attached to tungsten leads fused in standard cone joint, as shown in Figure 3. This forms a simple, sily removable heating unit, which will ensure a steady vigorous ullition of vapors even in liquids which are very prone to mping. An e.m.f. of approximately 6 volts will provide suffi- ent current for this heater.

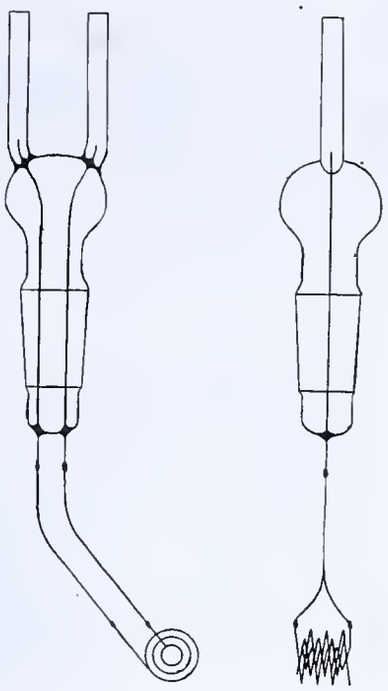

Figure 3. Internal Heating Element

The vapors generated within the boiler carry a stream of liquid to the Cottrell tube, and thus a liquid-vapor mixture is pumped to the thermometer. This mixture is then directed down- ards into the disengagement chamber, which consists of three ncentric tubes, through the inner of which the mixture enters. robably because of its downward momentum, the liquid is sepa- ted cleanly from the vapor at the bottom of this tube, and it ws away to return to the boiler. The disengaged vapors pass wards a short distance within the space between the inner and termediate tubes of the chamber and then escape to the outer cket, through which they pass downwards to the condenser. he disengagement chamber is thus jacketed with the issuing apors.

In systems of high boiling point, where radiation losses may ermit some condensation of the vapor during the short travel wards in the chamber, heat losses may be balanced by suitable ternal heating. Since both liquid and vapor which may be in ie outer jacket pass to the condensate system, neither slight eating nor cooling of the outer walls can introduce any errors in ie condensate composition.

The condenser and condensate trap present no new features. he condensate trap is essentially the same as that described by thmer (7); it has been provided with a standard-taper joint, to hich a further condenser may be fitted if desired, and this in turn connected to a suitable system for precise pressure regulation.

Overflow from the condensate trap is returned to rejoin the cir- ilating hot liquid through a small drip counter and capillary ibe. There is very little liquid holdup within the capillary, hich also serves to damp out fluctuations of the liquid level uring boiling. The combined liquid and condensed vapor reams re-enter the boiler through a small tube which passes to ear the bottom of the vessel, and the mouth is so placed and iaped that there is no tendency for vapor bubbles to pass out irough it.

In operation, the apparatus should be filled and the boiling ite adjusted so as to maintain the steady pumping of a mixture f liquid and vapor through the Cottrell tube, and the liquid level best kept fluctuating gently just above the capillary, in the rip counter. These conditions will be found satisfied by a wide range of boiling rates, but the volume of liquid within the boiler will require adjustment to within a few milliliters. A very rapid boiling rate will cause visible entrainment in the disengagement chamber; uneven or slow boiling allows vapor to pass up the Cottrell pump without entraining a stream of liquid, thus making this device ineffective. Strictly self-consistent data may be obtained over a wide range of operating conditions within these clearly defined conditions.

After allowing sufficient time of operation to ensure steady conditions within the apparatus, samples of the boiling liquid and condensed vapor are best taken simultaneously, without interrupting the heating in any way. The sample of boiling liquid should be collected in a small flask immersed in an ice bath, or it may be necessary in some cases to attach a small cooler to the sampling outlet (*6*).

It is hoped that data obtained using the apparatus will become available for publication at a later date.

ENTRAINMENT

With so short a distance within the disengagement chamber between the point of separation of liquid and vapor, the possibilities of entrainment are obvious, although none could be observed during trials of the apparatus over a wide range of boiling rates. The fine droplets which are characteristically found above the surface of some boiling liquids, due to collapsing vapor bubbles, appear to be entirely absent.

A simple test has been used to detect and quantitatively measure the entrainment. This test may be applied to any form of vapor-liquid equilibrium apparatus so far described, and may also be useful for the indication of evaporation of spray droplets from superheated surfaces. The boiler of the apparatus is charged with a 20% solution of potassium chloride, the condensate trap filled with distilled water, and the system allowed to boil at a constant rate for several hours, at the end of which the contents of the trap are tested for chloride by the standard opalescence method with silver nitrate. The results for a number of different boiling rates are given in Table I, and it is evident that the maximum entrainment obtained would not have any significant effect upon vapor-liquid equilibrium data.

Table I. Entrainment of Liquid in Vapor

Voltage Applied to Heater	Distillation Rate	Potassium Chloride in Liquid	Potassium Chloride in Condensate	Entrainment
Volts	*Ml./min.*	%	%	%
100	3.2	20	0.001	0.005
125	5.5	20	0.005	0.025
150	7.5	20	0.01	0.05

ACKNOWLEDGMENT

The author wishes to acknowledge the helpful criticism and assistance of John E. Cornish and R. F. Pickering during the development of this apparatus; also the provision of facilities for making and testing the apparatus by Beckers Pty., Ltd., of Adelaide, South Australia.

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Determination of 2,2-bis-p-Chlorophenyl-1,1,1-trichloroethane in Technical DDT

A Microscopical Method

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A microscopical method for analysis of mixtures of organic compounds is proposed, which depends on the influence of impurities on the rate of crystallization of the major component from the melt. The method is applicable to a large number of industrially important systems such as TNT or other explosives, pharmaceuticals, dye intermediates, or almost any organic reaction product. The application of this method to the determination of *p,p'*-DDT in technical DDT is described. It is possible by this method to analyze samples of technical DDT on a routine basis of one sample every 10 to 15 minutes with an accuracy of better than ±0.5% *p,p'*-DDT.

TECHNICAL DDT usually contains about 75% of the compound 2,2-bis-*p*-chlorophenyl-1,1,1-trichloroethane (*p,p'*-DDT), about 15% of the isomer 2-*o*-chlorophenyl-2-*p'*-chlorophenyl-1,1,1-trichloroethane (*o,p'*-DDT), and about 10% of other by-products of the reaction (*3*). Since the activity of DDT as an insecticide depends to a large extent on the amount of *p,p'*-DDT, it is important to be able to analyze quantitatively for this isomer. For this purpose, probably the most accurate of several methods of analyzing the technical material has been proposed by Cristol, Hayes, and Haller (*2*). This method is based on the insolubility of *p,p'*-DDT in 75% aqueous ethanol. The method is accurate (within 1%) and the technique is simple; it does, however, require temperature control and at least 8 hours for an analysis.

A microscopical method based on the rate of crystal growth of *p,p'*-DDT from the melt is described below. The basis for this analysis is the observation that rate of crystallization from the melt is a function of its purity (Figure 1). A pure compound will crystallize much more rapidly than the same compound in a less pure state. The nature of the impurity is not so important in most cases, since the mechanism of the decrease in rate of growth is almost entirely due to the interference of impurity molecules on crystal formation. If an adsorption effect were encountered where molecules of one impurity were adsorbed on the growing crystal faces, it would then be necessary to prepare the known rate curves on mixtures containing the same compounds that would be encountered in practice.

The technique of analyzing mixtures by determining the rate of crystallization of the principal component is applicable to a number of systems. It is limited, in general, to those mixtures that (1) do not decompose or sublime on melting; (2) can be supercooled to a constant known temperature before complete solidification; and (3) grow at a measurable rate at a convenient temperature. The method has the particular advantages of speed and the application to systems of isomers where no good absolute procedure for analysis exists. The method is applicable to a large number of industrially important systems such as TNT or other explosives, pharmaceuticals, dye intermediates, or almost any organic reaction product.

The basic assumption, made at the start of the present work, is that a technical sample of DDT containing 75% *p,p'*-DDT would behave in the same way as a mixture of 75% *p,p'*-DDT and 25% *o,p'*-DDT—in other words, that crystals of *p,p'*-DDT would grow at the same rate whether the melt contained only *p,p'*-DDT and *o,p'*-DDT or all the usual impurities and by-products were present. The final results have indicated the validity of this assumption.

A possible limitation of the method is the fact that compounds insoluble in the melt do not affect the rate of growth. A 50:50 mixture of *p,p'*-DDT and an insoluble component such as water, glass, dirt, or fibers would appear to be 100% *p,p'*-DDT by this method. The microscope would, however, show the presence of extraneous materials of this sort. On the other hand, extraneous solid impurities will not interfere with the analysis by this method except in so far as they interfere with the observation of the crystal front. This would occur only if the per cent solids was very high; in any case, the method of sampling suggested below would eliminate the possibility.

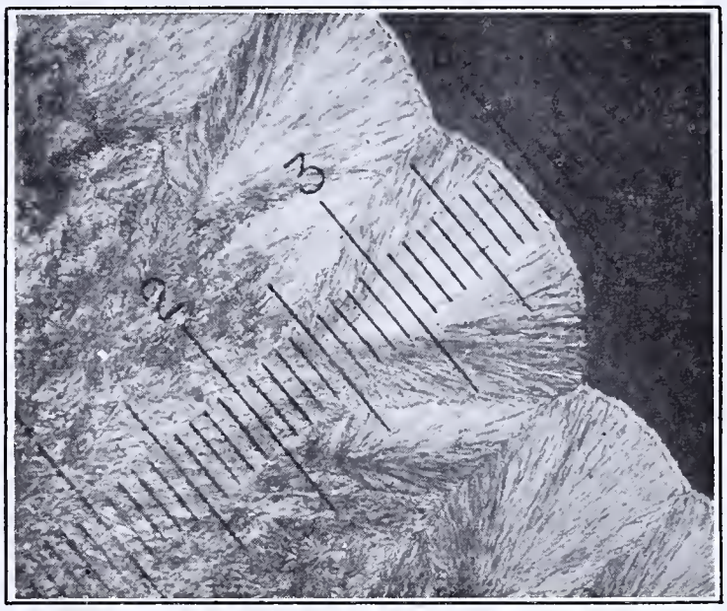
Since the rate of growth of crystals from the melt is also a function of the temperature, it is necessary to control, or be able to measure, the temperature. It is sufficient in an actual analysis to determine the temperature by supporting a thermometer with the bulb on the microscope stage near the preparation. It was necessary during the standardization work on this analysis to use a simple hot stage (Figure 2) to allow rapid changes in temperature and accurate control of any temperature desired. Although the dimensions of this hot stage are flexible, the difficulty of construction increases with the size of the chamber. The stage is heated with circulating liquid, usually water, whose temperature is controlled by a constant-temperature bath. Care must be exercised that the thermometer in the system records the temperature of the water as near the hot stage chamber as possible.

The rate of growth is determined using a microscope equipped with an eyepiece having a micrometer scale. The eyepiece micrometer scale must be calibrated in the usual way (*1*) with a stage micrometer scale before use. An eyepiece scale such that each division corresponds to about 5 microns or less is best. To increase the visibility of the crystal front of the *p,p'*-DDT crossed Nicols may be used (Figure 1). (In this case the eyepiece micrometer scale must be calibrated with the analyzer Nicol in place.)

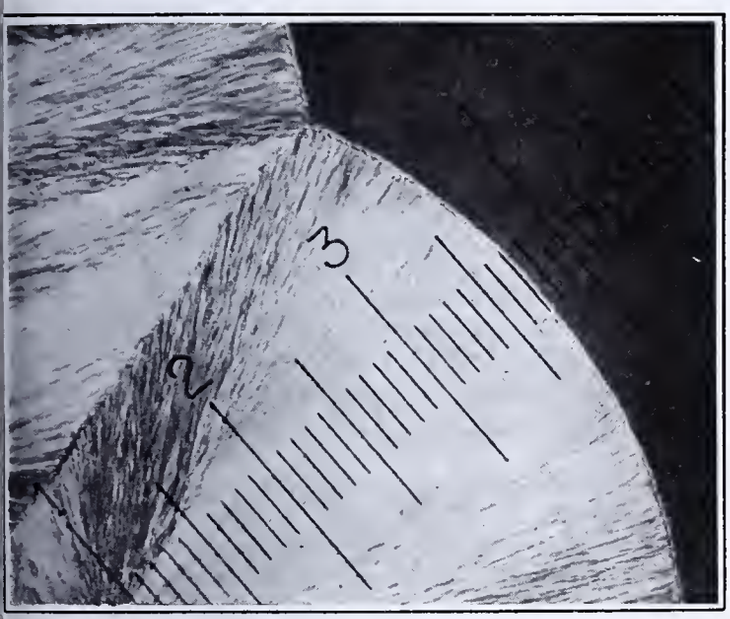
Certain precautions must be observed in order to obtain accurate results. The most important of these is sampling. Most industrial mixtures are not thoroughly mixed when considered microscopically. It is possible to obtain 50-mg. samples of technical DDT from a single container that vary as much as 6% in amount of *p,p'*-DDT. To eliminate this problem, which is common to all methods of microanalysis, a quick and efficient method of sampling must be used. This is provided for technical DDT by melting (avoid superheating above 120° C.) a 3- to 4-gram sample in an evaporating dish on a hot plate, stirring the liquid for a minute with a glass rod before removing the micro sample of one drop with the rod and placing it on a clean microscope slide. Other precautions almost as important as sampling are discussed below.

The hot preparation must be allowed to come to temperature equilibrium with the surroundings before being placed on the microscope stage (about 2 minutes) and must be allowed to come to complete equilibrium on the microscope stage (2 minutes) before a measurement is made.

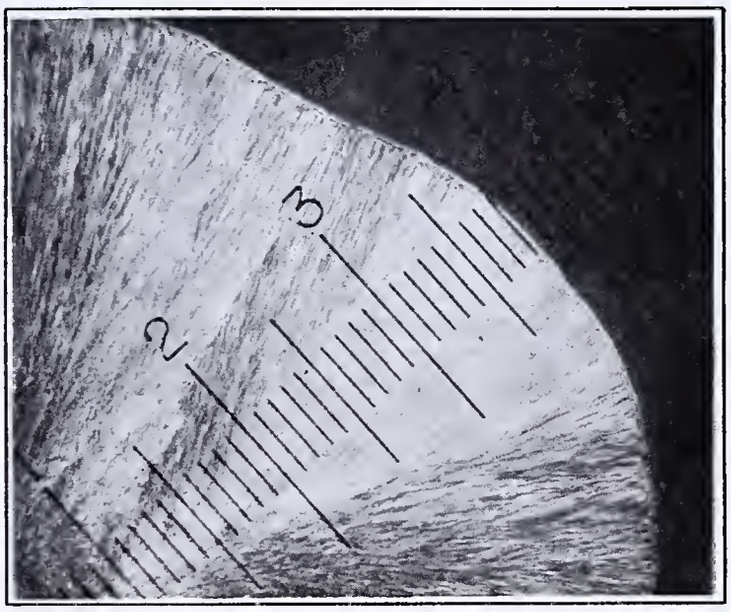
65% p,p'-DDT; 35% o,p'-DDT



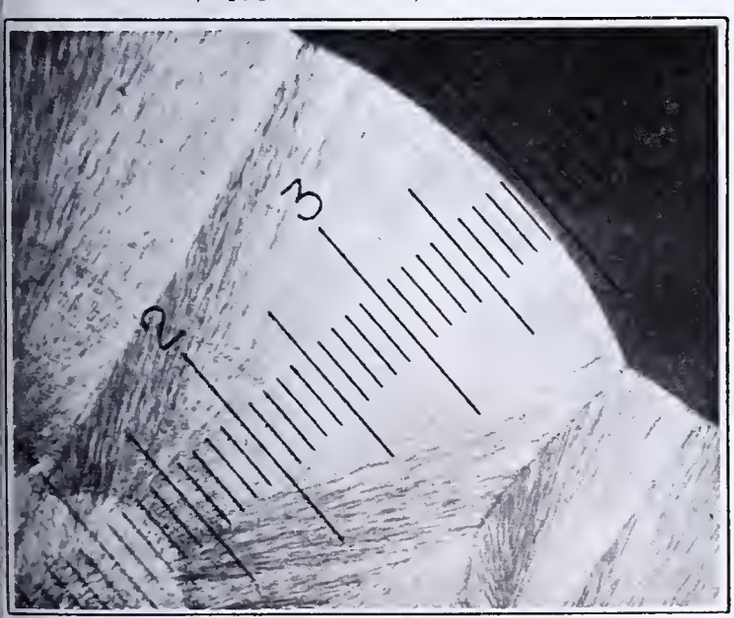
72% p,p'-DDT; 28% o,p'-DDT



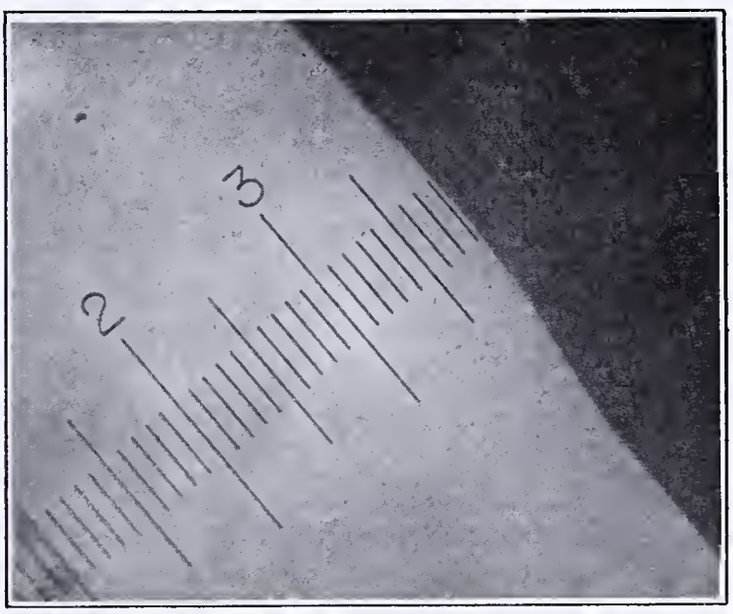
81% p,p'-DDT; 19% o,p'-DDT



82% p,p'-DDT; 18% o,p'-DDT



93% p,p'-DDT; 7% o,p'-DDT



100% p,p'-DDT; 0% o,p'-DDT

Figure 1. Growing Crystal Fronts of *p,p'*-DDT in Mixtures of *p,p'*-DDT and *o,p'*-DDT. × 200

To prevent heating of the preparation the light source must be as low in intensity as possible and still furnish easy observation of the crystal front. Normal illumination is probably satisfactory, especially if crossed Nicols are used. For best results a heat-absorbing filter—e.g., Aklo filter made by Corning—should

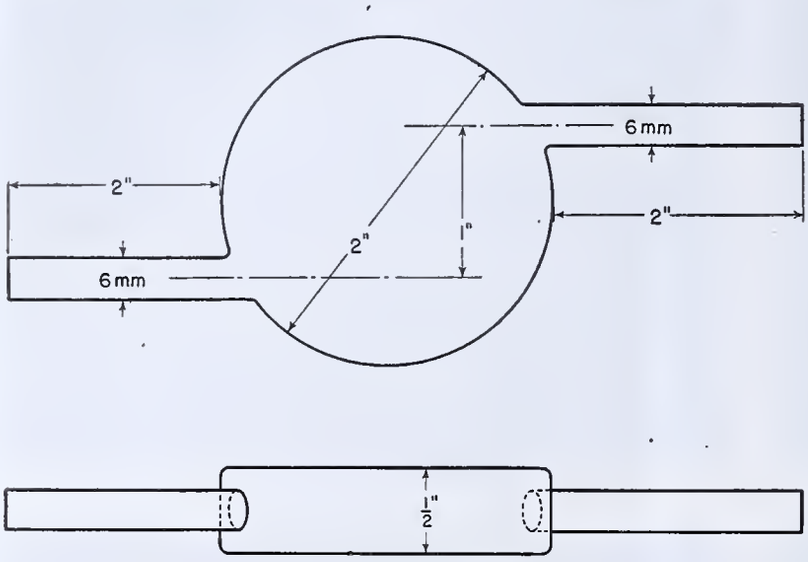


Figure 2. Diagram of a Simple Circulating Liquid Hot Stage

be used. It is also possible to determine a temperature correction by placing the thermometer bulb in place of the preparation for 1 to 2 minutes to obtain the temperature difference between the recorded value and the preparation. In general, a temperature difference of 0.1° C. is equivalent to a percentage difference of 0.3%; hence the temperature must be as accurate as possible. With normal diffuse illumination, crossed Nicols, and a heat-absorbing filter the heat gain in the preparation will be less than 0.1° C.

The measurement should be made for a time sufficiently long to give a reading of at least 10 eyepiece micrometer divisions. This will require as long as 15 minutes for very impure DDT (at low temperatures) but nearly pure DDT will require only 2 to 3 minutes.

The method would be more accurate and require less time if a permanent temperature control were available. This would be practical if a large number of routine analyses were necessary. For an occasional analysis or for a small number of analyses it is neither desirable nor necessary to go to the trouble to obtain temperature control. The results will be only slightly less accurate when no control is used (±0.5% compared with ±0.3%). Because of the heating effect of the illumination, the results without temperature control will tend to be high by perhaps 0.2 to 0.3%.

For the most accurate analysis of a number of samples of DDT the hot stage shown in Figure 2 should be used with a constant temperature of about 30° C. With this arrangement results having a precision of ±0.3% at the rate of one analysis every 10 minutes are easily possible.

PROCEDURE FOR ANALYSIS OF DDT MIXTURES FOR *p,p'*-DDT

A small sample (3 to 4 grams) is melted with as little superheating as possible on a hot plate (to avoid slight decomposition or volatilizing one or more components, the temperature should not exceed 120° C.). The melt is mixed by stirring for about one minute with a glass rod. One drop is then placed on a clean microscope slide and covered with a clean cover glass. The cover glass is pressed down firmly to give a thin film of DDT. If necessary, the preparation is seeded at the edge of the cover glass with a crystal of *p,p'*-DDT to initiate growth. The preparation is then allowed to cool for 2 minutes before placing on the microscope stage. At this same time a thermometer is placed on the stage with the bulb near the preparation. After 2 more minutes on the microscope stage the temperature is recorded and the crystal front focused with the eyepiece micrometer scale. The rate of growth of the *p,p'*-DDT is determined over a period of 5 to 10 minutes. The temperature is noted several times during the determination, so that the average may be used in determining the per cent *p,p'*-DDT. The rate of growth expressed in microns in 5 minutes and the average temperature are used in the graph (Figure 3) to determine the per cent *p,p'*-DDT.

If a temperature-controlled hot stage is used, little time is required to reach equilibrium. If the slide is usually a microscope slide cut in half 2.5 × 3.75 cm (1 × 1.5 inches), is placed on the hot stage with a drop of water between the slide and stage to increase the rate of heat flow, the rate of crystal growth can be measured as soon as the crystal front becomes well formed.

ANALYTICAL RESULTS WITH KNOWN MIXTURES OF *p,p'*-DDT AND *o,p'*-DDT

Mixtures of pure *p,p'*-DDT, melting point 108.5–109° C., with *o,p'*-DDT, melting point 74.0–74.5° C., have been studied by the procedure outlined above. These mixtures, ranging in composition from 65 to 100% *p,p'*-DDT, were melted and thoroughly mixed with a fine glass rod before being covered with a cover glass.

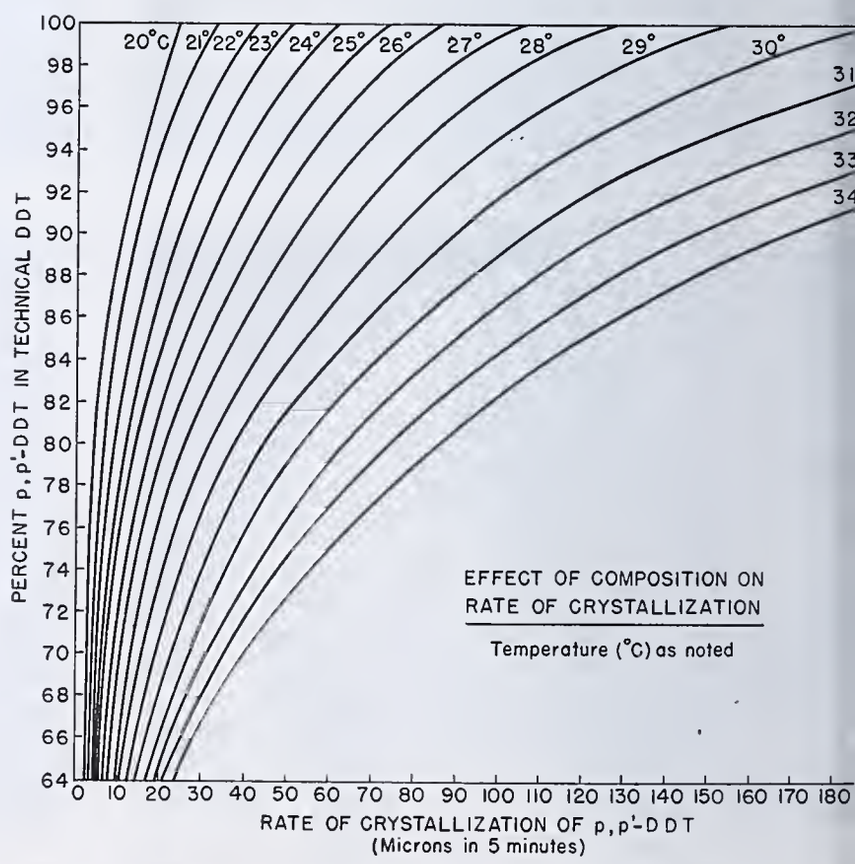


Figure 3. Rate of Growth of Crystal Front of *p,p'*-DDT as a Function of Purity of Mixture

The authors will be glad to furnish large reproductions of this graph to anyone requesting a copy

x of these preparations having compositions of 65, 72, 81, 82, , and 100% *p,p'*-DDT were used to determine the relation be- een rate of growth of *p,p'*-DDT and composition. These data re plotted, smoothed by graphical means, and replotted as own in Figure 3. All the data taken on known mixtures gave tes which checked to ±0.6 micron. This corresponds to about 0.3% *p,p'*-DDT at 30° C. and a mixture of 80% *p,p'*-DDT. ie error would be higher for lower temperatures and smaller rcentages of *p,p'*-DDT and less at higher temperatures and ncentrations of *p,p'*-DDT.

The results of the application of the method of analysis to three mmercially available samples of technical DDT are shown in ble I. A comparison of the rate of crystallization method of alysis with the recrystallization from 75% ethanol procedure of istol, Hayes, and Haller (*2*) is given by comparison of the alytical data by the two methods. The same samples were alyzed by both procedures and the data are presented in Tables and II. The two methods check very well with each other. ie data in Table II were obtained by as careful work as pos- le by an experienced analytical chemist. The data in Table were obtained with no temperature control and no particular ecautions or care not described completely above.

Table I. Analysis of Samples of Technical DDT by Rate of Crystallization of *p,p'*-DDT from the Melt

Sample I		Sample II	Sample III
75.7	75.2	73.1	71.8
74.8	75.2	74.7	72.1
76.0	74.6	73.4	71.5
75.3	75.6	73.1	72.8
75.2	74.7	74.1	71.8
74.9	75.4	73.8	72.4
75.3	75.1	...	...
75.9	...	...	...
erage	75.3	73.5	71.9
aximum deviation	0.7	1.2	0.9
erage deviation	0.3	0.5	0.4

It is possible with care to double the precision of the method, it this may not be advisable because the accuracy of the method not known. The accuracy is based on the analysis of known ixtures of *p,p'*-DDT and *o,p'*-DDT and the effect of the other -products and impurities on the rate of crystallization of *p,p'*- DT is assumed to be equivalent to an equal quantity of *o,p'*- DT. There is no way to check this hypothesis accurately. ie fact that the results agree within 1% with results obtained by e method of Cristol, Hayes, and Haller is significant only within e limits of error in that agreement—namely, 1%. Claims of curacy beyond that limit of less than 1% are unfortunately t justified.

ble II. Analysis of Samples of Technical DDT by Recrystallization from Saturated 75% Ethanol

Sample I	Sample II	Sample III
75.7	72.9	69.9
76.4	74.5	72.1
76.0	73.7	71.0

Further study of the rate data leads to an interesting and significant observation. The Arrhenius relation between the reaction velocity and temperature, in gaseous and liquid systems, is given by the equation

$$\log k = \frac{-E}{2.303R} \times \frac{1}{T} + B$$

Table III. Slope and Intercepts for Curves of Log Rate against Reciprocal Temperature

Per Cent Purity	$\frac{-E}{2.3R} \times 10^3$	$E \times 10^3$	Log B
100	7.27	33.2	26.3
93	6.87	31.5	24.7
82	6.56	30.0	23.5
72	6.24	28.6	22.1
65	5.78	26.5	20.3

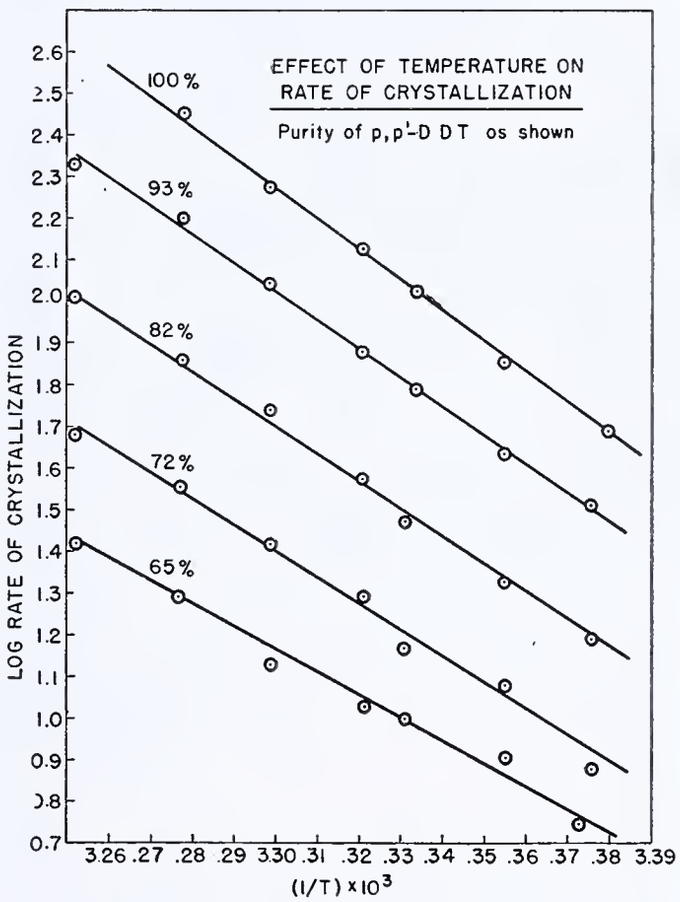


Figure 4. Result of Application of Arrhenius Rate Equation to Rate of Crystallization of *p,p'*-DDT

where the symbols have their usual significance. This equation is found to represent the data of the present work (see Figure 4) if k is considered to be the linear rate of crystallization. The Arrhenius constants, determined in the usual way, are presented in Table III.

The significance of the constant E (which is analogous to the activation energy in a chemical reaction) is probably based on the energy required for a molecule to assume a position relative to the crystal front convenient for crystallization. It would be interesting to study the effect of molecular symmetry on this energy constant. Presumably a symmetrical molecule would have less difficulty orienting itself on the crystal front than an unsymmetrical molecule and consequently should have a lower value for E.

If the rate of crystallization is plotted against the log of per cent impurity a series of curves of the linear type $y = m(x - a)$ is obtained (Figure 5). The common abscissa of these lines, estimated from the figure, is 1.625 (ca. 42% impurity). Calculated values of the slopes for the various temperatures are presented in Table IV.

A linear relation is found to exist between the log of the absolute value of the slope, m, and the reciprocal of the absolute tem-

Table IV. Variation of Curve Slopes (Figure 5) with Temperature

Temperature, °C.	Slope ($x - 1$)	Log Slope	$(1/T) \times 10^3$, °K.
34	270	2.431	3.257
33	236	2.373	3.268
32	202	2.304	3.279
31	168	2.225	3.289
30	139	2.143	3.300
29	114	2.053	3.311
28	95.9	1.982	3.322
27	80.6	1.906	3.333
26	67.8	1.831	3.344
25	56.9	1.755	3.356
24	46.5	1.667	3.367
23	38.8	1.589	3.378
22	30.8	1.489	3.390
21	24.8	1.394	3.401

perature (Figure 5, inset). This curve is represented by the equation

$$\log m = 26.37 - \frac{7.34 \times 10^3}{T}$$

T = absolute temperature

Substitution of $f(T)$ for m gives the complete equation

$$\log R = 26.37 - \frac{7.34 \times 10^3}{T} + \log (1.625 - \log c)$$

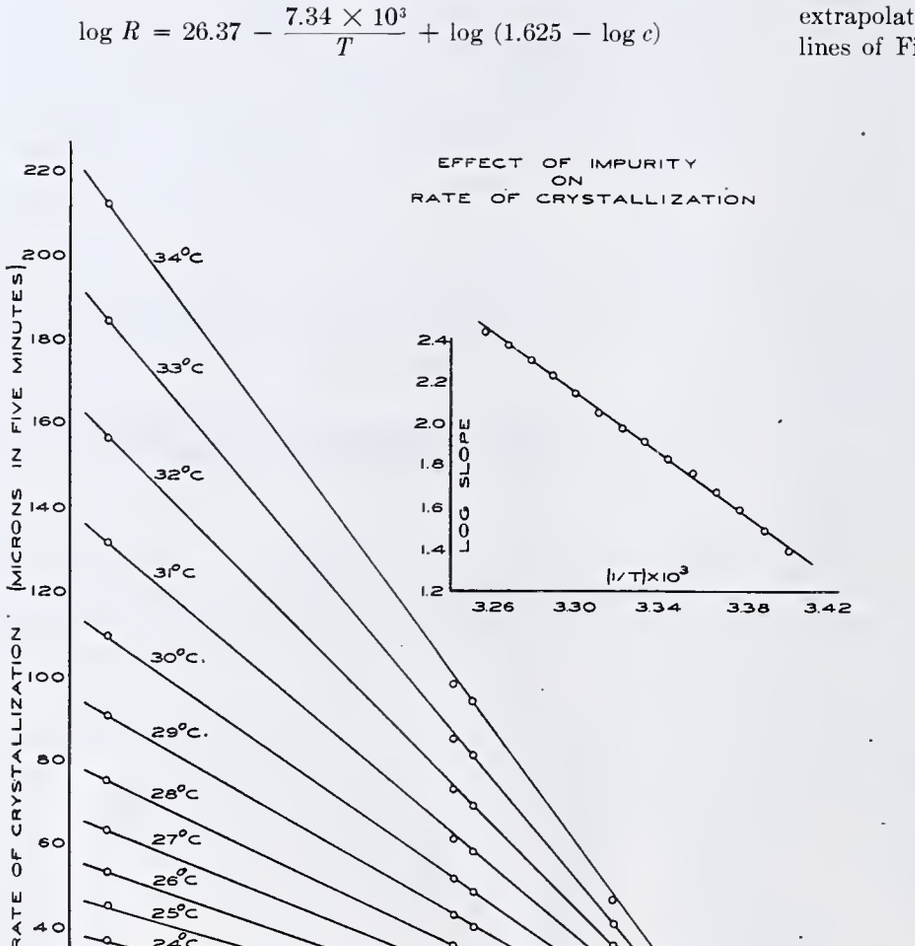


Figure 5. Rate of Crystallization *vs.* Log Per Cent Impurity

Table V. Comparison of Observed and Calculated Values

Impurity %	Temperature °C.	Rate (Observed)	Rate (Calculated)	Deviation from Observed Value %
2	27	90	60	33
3	27	83	69	17
4	27	72	78	8.3
5	27	72	74	2.8
5	33	218	224	2.8
6	27	67	68	1.5
6	33	200	204	2.0
7	30	109	107	1.8
7	33	184	187	1.6
18	24	17	17	0
18	31	61	62	1.6
19	26	23	23	0
28	22	5.5	5.4	1.8
28	25	10	9.8	2.0
30	27	13	12	7.7
30	33	36	36	0
32	27	12	12	0
32	33	30	29	3.3
34	27	10	7.4	26
36	27	9.	5.5	39

R = rate of crystallization, microns in 5 minutes
T = absolute temperature
c = per cent impurity in mixture

Table V indicates the range of validity of this equation.

The portions of Table V above 28 and below 7% impurity a extrapolations of the equation beyond the region covered by t lines of Figure 5. In the range between 5 and 32% impurit where the equation fits the data well, t calculated values for low rates are probab the more trustworthy, since it is difficult interpolate low rate readings on Figure 3 with 10 to 20%.

It is interesting to note that the expe mental rate of crystallization does not f to zero with approximately equal amounts pure and impure material, as the curves Figure 5 indicate. Dotted lines for two the curves show the true trend, which m be directed toward zero rate at 100% i purity. Although this region is theoretical interesting, it would be difficult and tim consuming to investigate experimentally.

ACKNOWLEDGMENT

The authors wish to acknowledge the ge erous donation of a sample of pure *o,p'*-DD by H. L. Haller of the Bureau of Ent mology and Plant Quarantine.

The analyses of technical DDT by t ethanol solubility method of Cristol, Hay and Haller were carried out by Mauri Kayner of the Armour Research Foundatio

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NOTES ON ANALYTICAL PROCEDURES

Iodometric Method of Analysis for Organic Peroxides

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BECAUSE of its accuracy, reliability, and general applicability, the iodometric method of analysis for organic peroxides has been utilized more often than any other. In spite of this wide usage, rarely have two groups of workers carried out the analysis in the same solvent. This is due primarily to the fact that the solvents used heretofore have not been completely satisfactory. Acetic acid has been used often, but, because of the rapid oxidation of iodide ions by atmospheric oxygen, the analyses must be carried out in an inert atmosphere of nitrogen (*1*) or carbon dioxide (*4*). Acetone is not very satisfactory because it reacts with iodine in the presence of water (*2*, *3*). Alcohols require no precautions against oxygen, but the liberation of iodine is slow and the solutions must be heated to incipient boiling (*3*). Most other solvents have been eliminated because of unfavorable side reactions or the low solubility of the reactants in the medium.

Table I. Iodometric Peroxide Analyses with Acetic Anhydride

Peroxide	% Active Oxygen after Standing 5 min.	15 min.	20 min.
Benzoyl peroxide (50-mg. sample)	6.60	6.60	6.60
	6.59	6.61	6.60
Peroxide in isopropyl ether	0.0493	0.0601	0.0601
			0.0601
Peroxide in *n*-butyl ether	0.0247	0.0325	0.0335
			0.0335
Peroxide in glyceryl trioleate	0.0335	0.0407	0.406
		0.0407	
Peroxide in diallyl phthalate	0.0401	0.0488	0.0488

The author has found that acetic anhydride is an excellent solvent for iodometric peroxide analyses. It is a good solvent for both peroxides and sodium iodide, it does not react with iodine, no precautions against oxygen are necessary, and the iodide-peroxide reactions are rapid when it is used as the solvent. In addition, the use of starch indicator (after dilution with water) makes possible the carrying out of accurate analyses on colored solutions.

ANALYTICAL PROCEDURE

The peroxide sample is placed in a glass-stoppered Erlenmeyer flask and 5 to 10 ml. of reagent acetic anhydride and 1 gram of powdered sodium iodide are added. After swirling to dissolve the iodide, the solution is allowed to stand for 5 to 20 minutes. Water (50 to 75 ml.) is added, the mixture is shaken vigorously for about a half minute, and the iodine is titrated with standard thiosulfate, using starch indicator. With very dilute solutions a slight positive correction of the starch end point may be necessary.

When polymers or other water-insoluble substances are present in the peroxide sample, it is advantageous to add a small amount of chloroform to the initial reaction mixture. After the subsequent addition of water, a two-phase system results which may be titrated in the usual manner to a starch end point or by observing the disappearance of the iodine color in the chloroform layer.

The acetic anhydride should not contain much acetic acid, since the rate of the atmospheric oxygen–iodide reaction is accelerated by acetic acid. Mallinckrodt reagent-grade acetic anhydride from a freshly opened bottle gave a blank of less than 0.01 ml. of 0.1 *N* thiosulfate after standing for 20 minutes with sodium iodide in air. Technical acetic anhydride has been used but it sometimes requires a small correction factor.

The method has been used most often with benzoyl peroxide (purified by adding three times a chloroform solution to cold methanol with stirring) and it has been found that 50-mg. samples can be correctly analyzed to within 0.2% (Table I). Other acyl peroxides gave results which were as reproducible as those with benzoyl peroxide. In the application of the method to peroxygen in ethers and ethylene derivatives, slightly longer reaction times were necessary to ensure complete reaction. A few typical examples are given in Table I.

Most substances do not interfere with the method, as is evident from Table II. Pyridine interferes when starch indicator is used but is harmless in its absence. The addition of phenols appears to result in slightly low results. Some ethylene derivatives react rapidly enough with iodine to cause trouble. Although the addition of acrylonitrile, methacrylonitrile, vinyl acetate, methyl acrylate, and allyl acetate resulted in nearly quantitative analyses, styrene and oleic acid reacted slightly and cyclohexene considerably under the experimental conditions. Thus, peroxygen analyses in the presence of ethylene derivatives—e.g., vegetable oils—require preliminary tests to determine the importance of iodine addition.

Table II. Effect of Added Substances on Analysis of Benzoyl Peroxide

(1 ml. of added substance, 50 mg. of peroxide, and 10 ml. of solvent)

Added Substances	% Peroxide by Analysis
None	99.8
Benzene, toluene	99.8
Carbon tetrachloride, chloroform	99.8
Ethyl acetate, ethanol	99.8
Cyclohexane, acetone	99.8
Nitrobenzene	99.7
Pyridine (without starch)	99.7
Acrylonitrile, methacrylonitrile	99.8
Vinyl acetate, methyl acrylate	99.7
Allyl acetate	99.4
Styrene	98.0
Oleic acid	97.0
Cyclohexene	80.0
Phenol	98.5
p-Cresol	98.5

ACKNOWLEDGMENT

The author wishes to express his appreciation to P. D. Bartlett for advice during the course of this investigation, and to the Pittsburgh Plate Glass Company for support of this research.

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Small All-Glass Ground-Joint Filters

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NOT long ago Craig and Post (*2*) described several small all-glass centrifugal filters in which the filtration takes place between the ground surfaces of a filter body and the filter stick. This paper describes an extension of this principle of filtering between ground surfaces, in which use is made of short standard-taper rough-ground joints. A preliminary description of one of these filters has been given (*1*). This development not only makes possible a much greater variety of filter designs, but at the same time confers several advantages on this type of filter in addition to those pointed out by Craig and Post. The filters can be used in the centrifuge in essentially the same manner as described by these authors, or they can be used in conventional setups for suction or positive pressure, but are not well suited for gravity filtration.

The ⅋ (1/10) filter-grindings have at least three distinct advantages over the very short, irregular-taper and low-angle grindings described by Craig and Post: The parts are interchangeable; the sticks always seat properly; and the sticks can be seated firmly so that they remain in place—no spring is required. An additional development consists of the variation of the "porosity" of the filters by controlling the fineness of the grinding—e.g., various porosities can be made by grinding with Carborundum powder of various sizes.

Several ground filters are illustrated in Figure 1. Various designs of filter bodies are represented by *H*, *E*, *A*, *C*, *I*, and *G*; of filter sticks by 1, 2, 3, 4, 5, and 6. *D* is a simple test-tube receiver with a heavy inside rim. *B* and *B'* are supporting tubes, the latter having a tiny vent. *F* and *F'* are funnels, the latter of light wall. Rubber supports (about 1 mm. thick) are used where indicated. Most glass parts were fabricated from standard-wall Pyrex tubing. The ground joints were made on the tubes with the aid of ⅋ graphite and cast iron tapers for shaping and grinding, or commercial ⅋ full-length joints were sealed to the larger tubing. Either before or after sealing, the joint was ground on a cast iron 1/10 taper with 150-mesh Carborundum-water mixture. The sealing operation destroys the top 10 to 20 mm. of the grinding on the full-length joints, leaving a ground surface of 10- to 15-mm. length. While finishing the lower end of this joint in the blow torch the bottom 5 mm. of grinding may or may not be destroyed. In any case control of the diameter and length of ground zone on both the body of the filter and the filter stick is simple when commercial ⅋ ground joints are used. [If commercial ⅋ "blank" (unground) joints could be used, the cost of these filters should be reduced.]

No doubt tapers other than 1/10 would also be suitable. Several porosities can be had for a given filter body by grinding each of several filter sticks with a different mesh of Carborundum. For general purposes 150-mesh Carborundum has been found satisfactory for all grindings. It is feasible to make these filter surfaces simply by grinding commercial joints against each other with Carborundum. This procedure is likely to destroy the interchangeability.

All the filters illustrated in Figure 1 are shown arranged for use in the 100-cc. brass cups of a No. 2 International centrifuge: *G*, *H*, and *I* can also be adapted for positive pressure, *H* and *I* for negative pressure. Other designs are readily constructed, such as heated or cooled, and larger or smaller capacity filters. Various styles of filter sticks can be used with various filter bodies.

The filter body, *I*, in conjunction with stick 3 (or 5, etc.) can be used in the same ways as a Corning 15 F fritted filter.

Combination *E4FE* is similar to one of Craig and Post. The weight of *E*4 is supported at the glass-to-glass contact of filter stick 4 and funnel *F*, between which liquid readily passes because they are not blown perfectly symmetrical. Although the weight of *E*4 has been as much as 30 to 40 grams, no breakages ha occurred at 1580 r.p.m. The ground neck of *E*, sticks 4 and 4', a funnel *F* should, of course, be made of reasonably heavy wa (about 1.5 mm. thick). The vented stick, 4', is useful if it is d sired to filter a hot solution. For handling hot solutions in vol tile solvents bottle *A* with vented stick 1 is better, because the is less evaporation loss.

Setup *G5B'F'E* is arranged for positive pressure or centrifug filtration of a solution preliminary to carrying out crystallizati or precipitation in *E*. A short-stemmed version of *I* can be us in place of *G*, and *C* can be the receiver in place of *E*. (T supports *B* and *B'* are better made of aluminum than of glas

Combination *H6E* is designed as an internally heated extracto Solvent can be distilled from *E* through 6 to a condenser a thence returned to *H*. Filtration can be hastened from time time by centrifugation. Filter sticks such as 6 can be ma shorter, and provided with a loose cap to prevent material fro falling into *E* when *H* is charged with material to be extracte Another arrangement similar to *H6E* has been described (To avoid breakage this design should not be centrifuged at spee much higher than 1000 r.p.m.

Setup *A1BC2* is shown in position for centrifugally filtering hot solution (to free it from debris) preliminary to crystallizatio In dissolving the material the solvent can be refluxed while A is hung from a hook through the eye of 1. The maximum volu which can be so handled is slightly less than half the total c pacity of *A*. During the crystallization in *C*, this bottle is ke closed by the filter stick by hanging the bottle with a hook throu the eye of 2. After equilibrium between crystals and solution established, the filtration is accomplished by centrifuging a rangement *C2D*. This closed "filter-bottle" design, *C*2, is esp cially advantageous for small-scale solubility determinations, a quantitative handling of recrystallizations or precipitations centrifugal filtration. Only the one tube must be handled on t balance. Before hanging in the balance the outside of *C* is wip if necessary with a wet cloth, washed with tap water, then wi redistilled acetone. With a similar glass tube as counterpo the weight is thus easily obtained accurate to ±0.1 mg. or bett The weighings are made with the bottle closed for the determi tion of the weight of solution and (after filtration) of the w crystals; and with the bottle open (the filter stick pushed in a the tube lying on the balance pan) for getting the weight of t dry (or drying) crystals. The rate of loss of methanol vap through a dry filter grinding ⅋ 10/8 is about 6 mg. in 24 hours 25° C.

The rate of filtration in the centrifuge from such a closed bot is only very slightly less than through an open filter if the bot is not over half full, the temperature of the contents is at or bel that of the centrifuge when the stick is pulled up, and the receiv

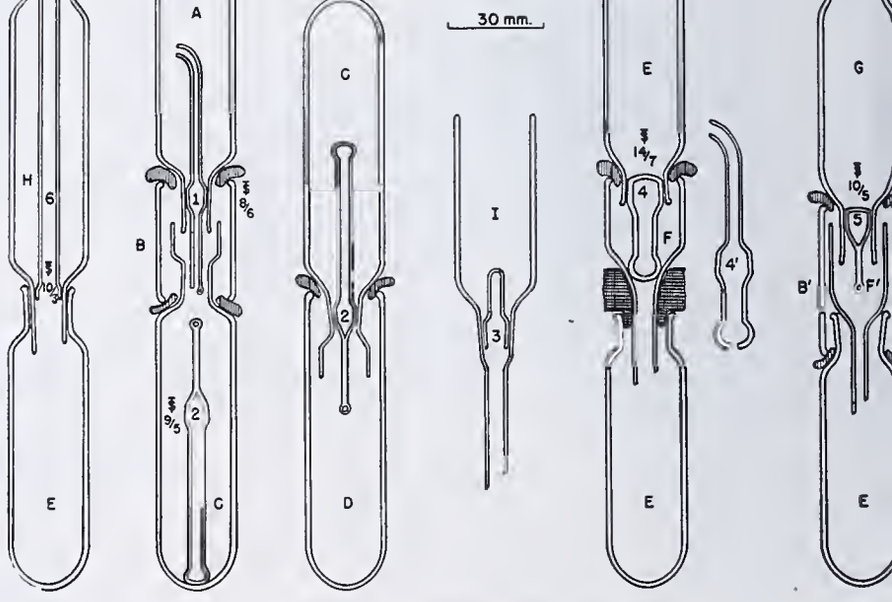

Figure 1. Ground-Joint Filters

has a vent—a tiny hole punched near the top of *D* suffices. (The vent is less helpful the smaller the volume to be filtered.) It is often possible to take advantage of nearly the full capacity of the bottle by removing some of the clear supernatant with a pipet before the filtration. Sometimes a few cubic millimeters of dry ice are added to a bottle three-fourths full just before pulling up the stick; the filtration is as rapid as through an open or vented filter.

The rate of filtration through the $\overline{\$}$ filters ground with 150-mesh Carborundum is very high, even though the length of the ground zone of contact is as much as 5 to 10 mm.

A filter such as *I* (Figure 1) having $\overline{\$}$ 10/8 was compared with a Corning 15 F fritted filter. Both were fitted with rubber collars and placed on receivers such as *D*, then charged with 10.0 of distilled water and centrifuged at 1580 r.p.m.; both filters let through 8.5 cc. in 2 minutes. If the receiver had no vent the ground filter was dry in a total of 3 minutes but the fritted filter held 1 cc. for at least 5 minutes; if the receiver had a vent both filters were dry in 3 minutes. The holdup of the ground filter was 0.05 cc., of the fritted filter 0.15 cc.

The retentiveness (in the centrifuge) of these two filters was comparable: both prevented the passage of any visible amount of barium sulfate when tested by the method of Scribner and Wilson (*3*); both readily allowed *Staphylococcus aureus* (1μ diameter) to pass through. (It is hoped that ground filters can be made which will retain the *Staphylococcus* and other bacteria. Experiments on this are being continued.)

There is room in the No. 2 International centrifuge (100-cc. brass cups) for a total length of filter plus receiver of 225 mm. when these have outside diameter of 25 mm. or less. Filters of type *C*2 weighing 30 to 50 grams have been repeatedly carried by receiver *D* without breakage at 1850 r.p.m. Still longer (larger capacity) filter bottles can be made for use with smaller capacity receivers in cases where much of the supernatant solution can be pipetted off before filtration. It would be wise to make them smaller and/or from lighter wall tubing if, for special purposes, speeds of 2000 r.p.m. or more are desired. The speed of 1580 r.p.m. has often given a holdup of only 20 mg. of mother liquor on 1 gram of moderate sized crystals.

SUMMARY

Small (up to 30 cc.) all-glass filters are described in which filtration occurs between standard-taper rough-ground surfaces. These filters are equal to or superior to small Corning F fritted filters in the following respects: they give equal rates of filtration, and have equal retentiveness; They have smaller holdup, are much easier to clean, and do not contaminate preparations with particles of glass. In adaptability of design they have distinct advantages over the filters described by Craig and Post. A new filter bottle is described which is particularly suitable for use in the centrifuge for determinations of solubility and for quantitative handling of recrystallizations and precipitations.

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Solution Intake Unit for Improved Operation of the Flame Photometer

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IN USING a recent model of a commercial flame photometer, annoying drift and fatigue of the phototube were encountered (*1*). As a result one standard solution had to be run alternately with the sample solution tested. An improved solution intake unit has been added to the atomizing system which increases the speed and accuracy of operation.

Figure 1 shows the original solution intake and the improved unit. With this double capillary intake and the three-way stopcock, the standard solution can be left in the system continuously, changing only the beaker containing the sample solution, which is alternated with a beaker of distilled water for flushing the atomizing system. A sample reading can be made very quickly, after setting the instrument on the 100-mark with the standard solution. In other words, the time allowed for drifting of the phototube has been kept at a minimum. However, the most important improvement is the elimination of the extra operation of handling the standard solution which now stays continuously in the system.

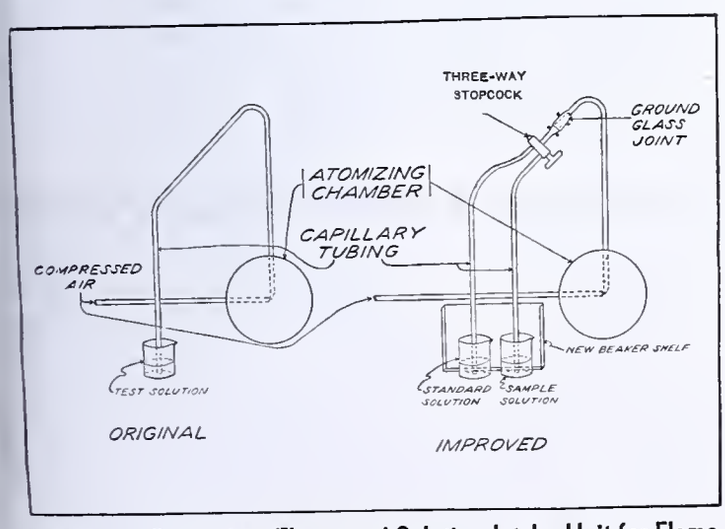

Figure 1. Original and Improved Solution Intake Unit for Flame Photometer

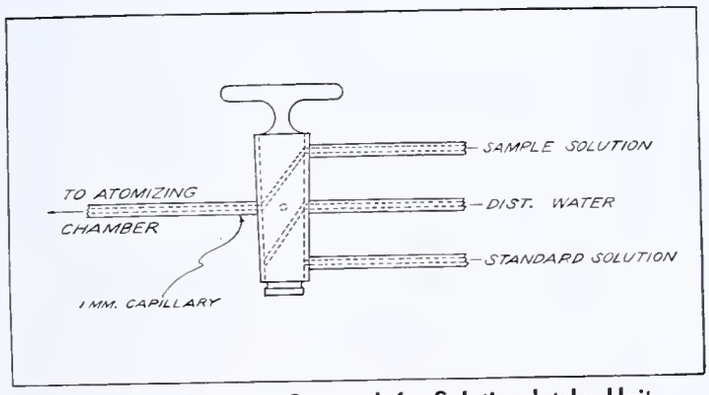

Figure 2. Four-way Stopcock for Solution Intake Unit

A further possible improvement is shown in Figure 2, where a four-way stopcock allows a beaker of distilled water to be added for flushing the atomizing system. This distilled water remains continuously in the system like the standard solution; thus the operator need handle only the beaker containing the sample solution.

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The assistance of Jane L. Showacre in trying out the new unit is appreciated.

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Cleaning Laboratory Glassware

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THE device illustrated in Figure 1 has proved particularly useful in cleaning laboratory glassware with corrosive fluids such as chromic acid or sulfuric-nitric acid mixtures. It is also useful for drying glassware with organic solvents such as acetone, or for making collodion membranes in test tubes. The unit is constructed of Pyrex and can be heated directly if hot cleaning solution is desired.

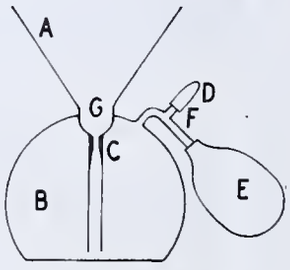

Figure 1. Apparatus

A, 4 to 5-inch funnel; *B*, 0.5 to 1.0-liter flask; *C*, constriction in 10-mm. tube, internal diameter, 2 to 3 mm.; *D*, medicine-dropper bulb; *E*, 60-ml. rubber bulb; *F*, 8-mm. tubing; *G*, throat, about 18-mm. internal diameter

In operation, a flask, beaker, etc., is inverted inside the funnel, *A*, directly over the throat, *G*. Abrupt pressure on the rubber bulb, *E*, produces a strong jet of fluid which floods the walls of the vessel and then drains back into the funnel. On release of the bulb, the excess fluid is sucked back into chamber *B*. Flasks, test tubes, beakers, bottles, and graduates are acid-cleaned in this manner very rapidly. Flasks, for example, have been acid-treated at a rate of over 750 per hour.

If the vessels being cleaned contain a large amount of reducing material which can wash back into the chamber, the cleaning solution will deteriorate. However, in normal use this does not occur. Most of the cleaning action probably occurs in the film of acid left in the flask. That this film of acid is sufficient to clean the glassware thoroughly, if the flasks are left standing for a short time, is indicated by the fact that after rinsing with distilled water, interference rings form within 1 or 2 minutes.

The small medicine-dropper bulb, *D*, is removed when the chamber is being filled. To prevent accidents, it is wise to remove *D* whenever the device is not actually in use.

BOOK REVIEWS

Qualitative Organic Microanalysis. *Frank Schneider.* iv + 218 pages. John Wiley & Sons, Inc., 440 Fourth Ave, New York 16, N. Y., 1946. Price, $3.50.

In this short book the author has attempted to create a "guide to the microtechniques required for the identification of organic compounds". The first half of the manual consists of a compilation of numerous published micromethods applicable to fundamental organic operations. The latter half is an abbreviated treatment of the problems of qualitative organic analysis along with laboratory directions for preparing about 30 derivatives.

Techniques of separation and the handling of mixtures are not adequately treated by the author, and consequently he had no reason to discuss some of the newer practical micromethods for this work. One thinks particularly of diffusion analysis and chromatography. The latter might have been mentioned under the heading "purification of the sample".

Since many difficult industrial problems in the field of organic identification are being solved every day by methods of chemical microscopy, it would seem that the author ought to have offered more encouragement to the reader in the use of the microscope as a tool in organic analysis. He mentions the scarcity of optical data for organic compounds; yet he does not list even the most valuable literature sources of such data now in existence. For instance, there is no mention of Winchell's recent book which lists optical data for over a thousand organic compounds, or of the Barker tables which are being published in England.

The first portion of the book dealing with organic operations is abundantly provided with diagrams of the microapparatus recommended. Such a collection should prove a useful reminder to even the experienced organic chemist who may have seen them before. Unfortunately some of the pieces drawn are too large to be practical for true microwork—i.e., 30 to 100 mg. of substance—being more suitable for "submacro" work, 0.5 to 1.5 ml. Yet this criticism does not detract from the usefulness of the manual.

The book is free from typographical errors and is attractively printed in a medium-sized type suitable for student use. On pages 121 and 122 the author uses "crystalline form" indiscriminately when he means "crystal habit" in some instances. Another slip is the frequent use of the word "microscopic" for the more appropriate "microscopical".

In spite of our criticism the book fills a useful purpose and is a pioneer in the field which it covers. J. A. Kuck

Standard Methods for Testing Petroleum and Its Products. 7th edition. 550 pages. Institute of Petroleum, 26 Portland Place, London, W.1, England; and American Society for Testing Materials, 1916 Race St., Philadelphia 3, Pa. Price, 15s. or $3.25.

An added feature is a list of new methods, revisions of methods and specifications, and withdrawals. Two new methods have been added and 21 standards and two specifications have been effected by various changes. The conversion tables for petroleum oils have been withdrawn.

One of the new methods is a modification of a test for the determination of carbon disulfide in tar and its products to be applied to petroleum products. The other new method is intended to detect the coagulation of bitumen emulsions at low temperatures.

The method for knock rating of aviation fuel (weak mixture) has been rewritten to correspond to the A.S.T.M. format. Several changes have been made in the knock rating methods for experimental fuels over 100-octane number and for motor fuel.

Changes in procedure and interpretation of tests are few. A larger sample is permitted in the test for low acidity. Copper strip tests for corrosive sulfur are to be interpreted as one of six classifications of color change. Methods for the determination of tetraethyllead in motor fuel by bromination and hydrochloric acid are modified. The bromination method is not applicable directly to blends containing alcohol and is not recommended for fuels containing a high percentage of unsaturated hydrocarbons.

Modifications are indicated in apparatus for the following methods: distillation of liquid asphaltic bitumen, drop point of greases, flash point (closed) by means of the Pensky-Martens apparatus, penetration of bituminous materials, recovery of asphaltic bitumen from asphalts, residue on evaporation of kerosene and tractor fuel, sludging value of transformer oil, softening point of asphaltic bitumen (ring-and-ball method), viscosity by the Redwood apparatus, and water and sediment by means of centrifuge.

Editorial changes have been made in methods for ash of petroleum products, color by means of the Lovibond tintometer, distillation of crude petroleum, and sampling petroleum and petroleum products.

The title of the specification for wartime hydrometers now has an emergency status. Modifications in the specifications for wartime standard thermometers are more extensive. F. E. Bucha

Abstracts—Spectrochemical Analysis

The Ohio Valley Spectrographic Society announces the publication and distribution of Collected Abstracts Published during 1945 on Spectrochemical Analysis, compiled by Edwin S. Hodge. The 64-page lithographed booklet contains verbatim copy of all the abstracts published in 1945 by *Chemical Abstracts* on articles dealing with spectrochemical analysis. Over 80 references are cited, covering all the latest reported developments in the science, including analysis of alloys, direct-reading instruments, and other progress. The subject matter is arranged according to twelve topics and is well indexed.

The book is available from the Ohio Valley Spectrographic Society, Engineers Club Bldg., Dayton, Ohio, at $1 per copy if cash is sent with order; $1.25 when billing is required.

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WALTER J. MURPHY, EDITOR

Use of Control Charts in the Analytical Laboratory

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Control charts are useful in the testing laboratory for comparing the over-all variability of test data with the average variability of small groups of the data, and are simpler to understand than complicated statistical methods of analysis of variance. Operational meaning of control charts depends on the manner in which arbitrary small groups are chosen.

STATISTICAL methods have been used for a long time to present the average of test method results. However, as Fisher has pointed out (*5*), the variation among test results usually has not been an object of study, but has been recognized rather as a troublesome circumstance which detracted from the value of the average.

The estimates of test method precision which analysts do present are often not correct because they are made on the basis of a small amount of data covering only a short period of time. In many cases, a great deal of effort has been taken to eliminate all assignable causes of variation while the precision of the test method is being studied, even though it would not be desirable or even possible to do so when the test method is used for routine control purposes. W. S. Gosset, who published under the pseudonym of "Student", stated all this aptly when he observed (*17*) that an analyst who wishes to impress his clients will arrange to do repetition analysis as nearly as possible at the same time, but if he wishes to diminish his real error he will separate them by as wide an interval as possible.

The application of statistical methods to industrial manufacturing problems has been pioneered in the United States by Shewhart (*14*). During the war, the War Production Board sponsored some thirty-three short courses on the industrial application of Shewhart's methods in which almost two thousand men were trained. With the coming of these "quality control" methods into chemical-process manufacturing, it will be increasingly important for analysts to be able to determine how much of any observed process variation is to be ascribed to the test procedure itself. The control chart method of Shewhart is probably the most practical method of studying the precision and accuracy of routine test methods, and several authors have already mentioned its application to the study of chemical test procedures (*4, 7, 8, 10, 20*).

No attempt will be made here to develop the mathematics upon which control charts are based; and only a brief reference will be made to the simple arithmetical calculations involved because this part has been adequately presented in many publications (*1, 2, 6, 12–16*). An attempt will be made to show the operational meaning which can be put into control charts when applied to problems in the analytical laboratory.

MAKING A CONTROL CHART

A control chart may be described as a graphic presentation of test data in such a manner that the variability of all the results is compared with the average variability within (arbitrary) small groups of the test results. The chart is said to show evidence of "control" when there is no more variation throughout the entire set of results than corresponds, statistically, to the average variation within the (arbitrary) small groups.

In order to illustrate the preparation of a control chart, use can be made of a comprehensive experiment made by Shewhart (*14*), in drawing marked chips from a bowl. In his experiment, 998 chips were marked as shown in Figure 1, and 1000 groups of four chips were drawn with replacement from the well-mixed bowl. The averages and ranges (difference between highest and lowest) of the first fifty drawings are plotted in Figure 2. The grand average was −0.08 and the average range of four was 2.03. It can be seen that this graph of random drawings is similar to a graph for routine test results, in that both the averages and ranges fluctuate and the question immediately arises as to whether any limits for these variations can be set up.

Table I can be used to set up these limits as follows: The upper

Table I. Factors for Computing 3-Sigma Control Limits

No. of Observations, n	Chart for Individuals, Factors for Control Limits		Chart for Averages, Factors for Control Limits		Chart for Standard Deviations			Chart for Ranges		
	Av. standard deviation, I_1	Av. range, I_2	Av. standard deviation, A_1	Av. range, A_2	Factor for central line, c_2	Factors for control limits B_3	B_4	Factor for central line, d_2	Factors for control limits D_3	D_4
2	5.32	2.66	3.76	1.88	0.5642	0	3.66	1.128	0	3.27
3	4.15	1.77	2.39	1.02	0.7236	0	2.69	1.693	0	2.57
4	3.76	1.46	1.88	0.73	0.7979	0	2.33	2.059	0	2.28
5	3.57	1.29	1.60	0.58	0.8407	0	2.13	2.326	0	2.11
6	3.45	1.18	1.41	0.48	0.8686	0	2.00	2.534	0	2.00
7	3.38	1.11	1.28	0.42	0.8882	0.10	1.90	2.704	0.08	1.92
8	3.32	1.05	1.18	0.37	0.9027	0.17	1.83	2.847	0.14	1.86
9	3.28	1.01	1.09	0.34	0.9139	0.23	1.77	2.970	0.18	1.82
10	3.25	0.97	1.03	0.31	0.9227	0.27	1.73	3.078	0.22	1.78

Control limits for individual observations $= \bar{\bar{X}} \pm \frac{3\bar{\sigma}}{c_2} = \bar{\bar{X}} \pm I_1\bar{\sigma} = \bar{\bar{X}} \pm \frac{3\bar{R}}{d_2} = \bar{\bar{X}} \pm I_2\bar{R}$

Control limits for averages of groups of $n = \bar{\bar{X}} \pm \frac{3\bar{\sigma}}{\sqrt{n}c_2} = \bar{\bar{X}} \pm A_1\bar{\sigma} = \bar{\bar{X}} \pm \frac{3\bar{R}}{\sqrt{n}d_2} = \bar{\bar{X}} \pm A_2\bar{R}$

Control limits for standard deviations $= \left(1 \pm \frac{3}{\sqrt{2n}\,c_2}\right)\bar{\sigma} = B_3\bar{\sigma}$ to $B_4\bar{\sigma}$

Control limits for ranges $= \bar{R} \pm 3\sigma_R = D_3\bar{R}$ to $D_4\bar{R}$

$\bar{\bar{X}}$ = grand average; $\bar{\sigma}$ = average standard deviation; $\bar{R}$ = average range

With the exception of factors in columns for I_1 and I_2, this table is taken from (*1*) Table I, page 50; the formulas are explained on pages 52 and 53.

and lower limits for range variation are equal to the average range, 2.03, multiplied by the factors 0 and 2.28 (which are found in columns D_3 and D_4 corresponding to a group size of four under chart for ranges). This gives a lower limit of 0 and an upper limit of 4.6 for the variation of ranges of four drawings. Ranges greater than 4.6 (and less than 6.0) are possible but they should be observed only one or two times per thousand drawings by chance alone.

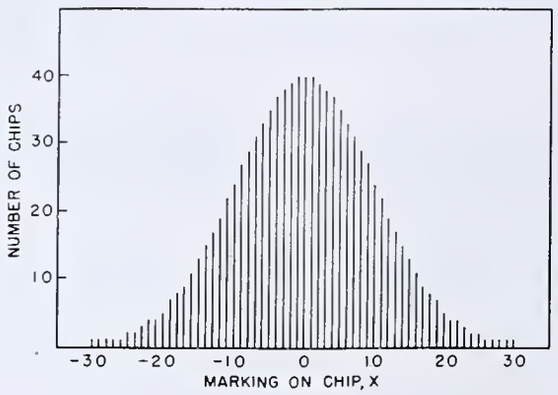

Figure 1. Distribution of 998 Chips in Shewhart's Sampling Experiment

Average, 0.0
Standard deviation, 1.007

In a similar manner, the limits of variation for the averages of four about the grand average are equal to the average range, 2.03, multiplied by the factor 0.73 (which is found in column A_2, corresponding to a group size of four under chart for averages). This gives the limits -0.08 ± 1.48 for the variation of averages of four drawings. A summary of these calculations is given in Table II.

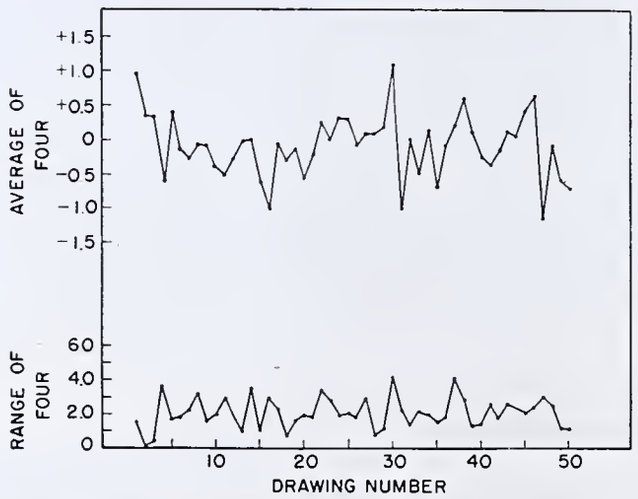

Figure 2. Two Hundred Drawings from Shewhart's Bowl of Chips in Groups of Fours

These so-called "control limits" are drawn in as shown in Figure 3, and it can be seen that the ranges and averages are all within their control limits, as of course they should be, if Shewhart's experiment was carried out properly. The control chart tells us graphically that there was no more variation among the fifty averaged values than corresponds, statistically, to the average range of four individual chips drawn at one time. If an infinite number of drawings were tabulated and summed up, the results would make a distribution curve like that shown at the right of Figure 3; the control limits correspond, approximately, to the points on either side of the grand average where this distribution curve approaches the zero base line.

The table of factors can be used to estimate control limits for

Table II. Summary of Calculation of Control Limits for Shewhart's Drawing Experiment

Drawing No.	X_1	X_2	X_3	X_4	$\bar{X}$	R
1	1.7	0.2	1.4	0.5	0.950	1.5
2	0.3	0.3	0.4	0.4	0.350	0.1
3	0.4	0.4	0.1	0.4	0.325	0.3
4	−1.8	−0.9	−0.6	1.7	−0.400	3.5
5	0.5	−0.7	0.8	1.0	0.400	1.7
...	...	...	...	...	...	...
..	...	...	...	...	...	...
..	...	...	...	...	...	...
46	0.6	2.2	0	−0.2	0.650	2.4
47	0.5	−0.7	−1.9	−2.5	−1.150	3.0
48	0.3	−1.5	1.0	−0.1	−0.75	2.5
49	−1.2	0	−0.8	−0.5	−0.625	1.2
50	−0.7	0	−1.0	−1.1	−0.700	1.1
			Sum		−4.00	101.4
			Av.		−0.08	2.03

Control limits for $R = (0 \times 2.03)$ to $(2.28 \times 2.03) = 0$ to 4.6
Control limits for $\bar{X} = -0.08 \pm (0.73 \times 2.03) = -1.56$ to $+1.40$
For complete data see (*14*) Table A, p. 442.

ranges, standard deviations, and averages of small groups of test results as well as for the individual results themselves. When these limits are drawn into the graph, the resulting control chart shows at a glance whether there is more variation among the groups (or among all the individual results) than corresponds, statistically, to the average variation within the groups. If excessive variation is found to be present, the control chart will often help to find its cause.

CHECKING PERFORMANCE OF ROUTINE TEST METHODS

Many laboratories maintain a "controlled sample" which is used at regular intervals to check the performance of a routine test method. The control chart in Figure 4 shows such test results for a simple viscometer. The controlled sample is run every day just before the production samples are tested. The five weekly results for the controlled sample are grouped in order to estimate control limits. The lower graph for weekly ranges indicates no variations greater than are to be expected by chance alone, and the upper graph shows that there is no more variation among the individual results from week to week than corresponds, statistically, to the average variation of any one week. These limits of test variation are satisfactory to the production department.

This kind of chart is invaluable when new test operators are being trained or when it becomes necessary to change the controlled sample. The chart also stops effectively most of the arguments between the testing laboratory and the production department over the validity of production test results which are not as they should be.

Sometimes it is possible to keep a satisfactory check on a rou-

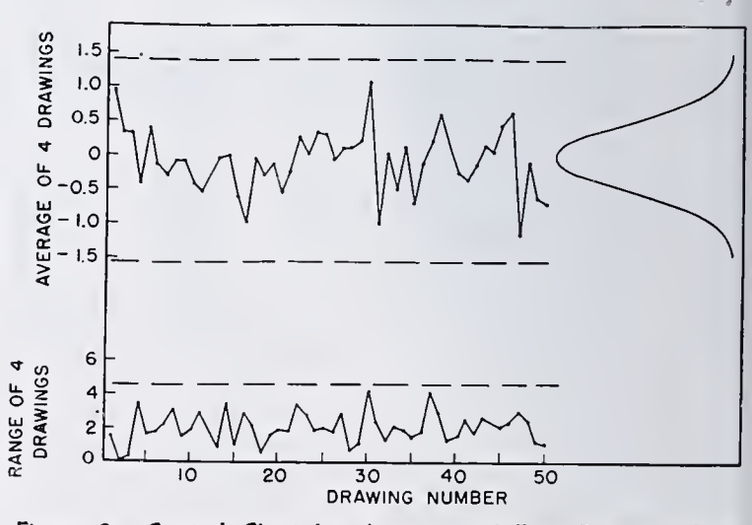

Figure 3. Control Chart for Averages of Four Drawings from Shewhart's Bowl of Chips

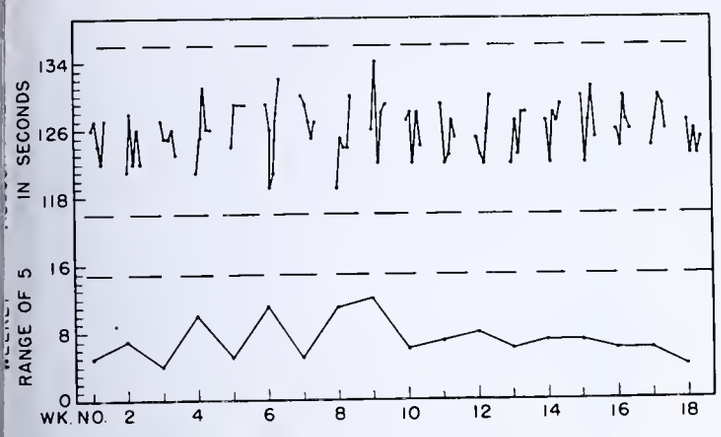

Figure 4. Control Chart for Viscosity of a Standard Sample

ine test method without the use of a controlled sample. Figure 5 hows a control chart for the moisture content of production dis- illation lots of an organic solvent. The lower graph shows that he differences between two consecutive lots of the solvent do not luctuate more than corresponds to chance alone. The upper raph for individual lots indicates that there is no more variation rom lot to lot than corresponds, statistically, to the average dif- erence between two consecutive lots. This control chart com- ines the variations of the production department and the testing aboratory, but the chart still serves a useful purpose in the labo- atory as long as all results are within their own control limits and he control limits are within the specification limits (in this case) to 0.6% moisture).

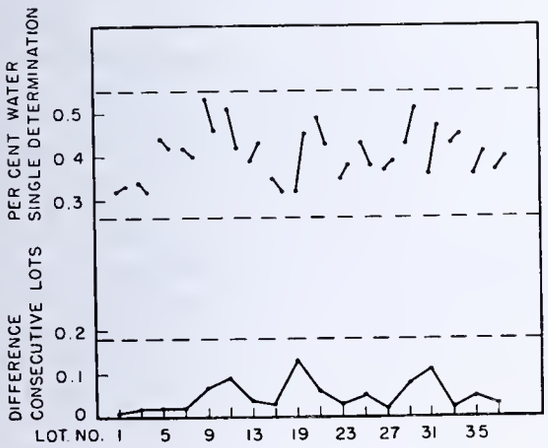

Figure 5. Control Chart for Routine Moisture Determinations on Production Lots of an Organic Solvent

When the routine test results show lack of control, the analyst checks his reagents and then verifies his results on replicate samples from the suspected lot. The use of such a control chart sometimes makes it possible to reduce the amount of routine testing when a production process is not giving trouble. As soon as lack of process control is indicated, the amount of routine testing can be increased immediately.

COMPARING MERITS OF ALTERNATIVE TEST PROCEDURES

It is often desirable to compare the merits of alternative test procedures, and a control chart will usually help to do this in a manner which is both objective and quantitative. Figure 6 shows the results of a comparison between the so-called method of single swings and the more commonly used multiple-swing method for weighing on the analytical balance.

Four individual comparisons of two 1-gram and two 100-gram weights were made on ten different days using the two methods. With one exception, the daily variation (standard deviation) does not fluctuate significantly greater than it should by chance alone and, with four exceptions, the individual weighings are all within their control limits.

No assignable causes were found which would account for the occasional lack of control for individual weighings, but they may be the result of air drafts or faulty manipulation in releasing the beam rests. A control chart for daily averages would indicate some evidence of more variability in the weighings from one day to another than corresponds to the average daily variability. However, there is no evidence that either of the two weighing procedures is superior to the other for weighing loads up to the full capacity of the balance. One important thing to note in favor of the method of single swings is the fact that it requires about one quarter as much time as the multiple-swing method.

Mention has been made of control charts for individual test results and for averages of test results. It is always better, from a statistical point of view, to chart averages rather than the individuals themselves. However, there are many occasions in the analytical laboratory where single observations rather than averages are used or reported. Thus, only rarely would weighings be made in replicate and the average weight used or reported. In such cases the individuals can be charted, although they must be grouped arbitrarily in order to estimate control limits. It is often desirable to chart averages as well as individuals because the chart for averages gives valuable information about the general pattern of a set of test results, even though it is only the individual results which are reported or used.

CONTROLLING ERRORS OF CALIBRATION OF TESTING EQUIPMENT

The calibration of testing equipment is often plagued with the errors of the calibration method itself, and control charts will offer help in such cases. Figure 7 shows the results of calibrating nine burets by weighing the water delivered at a known temperature to the 39-, 40-, and 41-ml. marks. The lowest graph shows the range of three consecutive individual tests made for each mark, and it can be seen that there are no variations from range to range greater than correspond to chance alone, although the upper limit of 0.03 ml. seems high. These range variations were not the result of insufficient drainage time, and they seem to represent real differences in the amount of water held up on the walls of the buret.

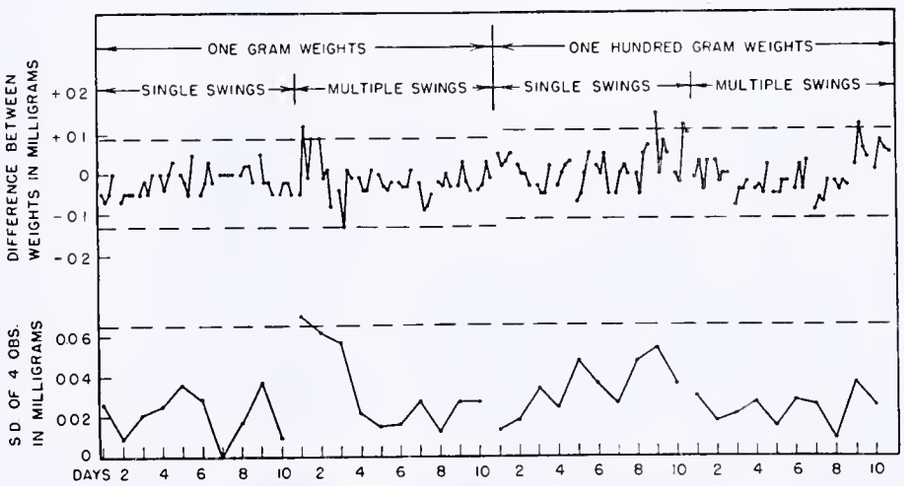

Figure 6. Control Chart for Comparing Weighing Methods

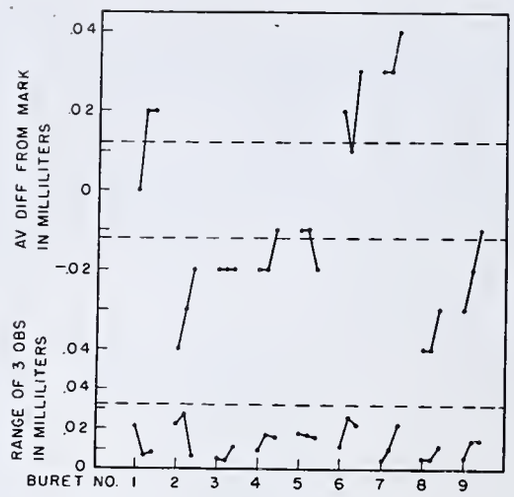

Figure 7. Control Chart for Buret Calibrations

Each buret checked at 39-, 40-, and 41-ml. marks

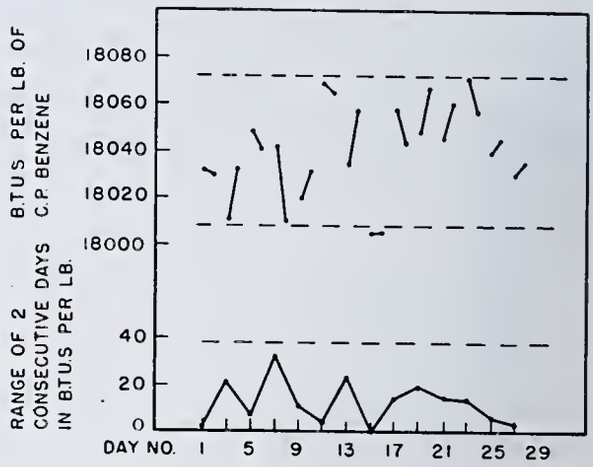

Figure 8. Control Chart for Heat of Combustion of C.P. Benzene

Data of Dean, Williams, and Fisher (*3*)

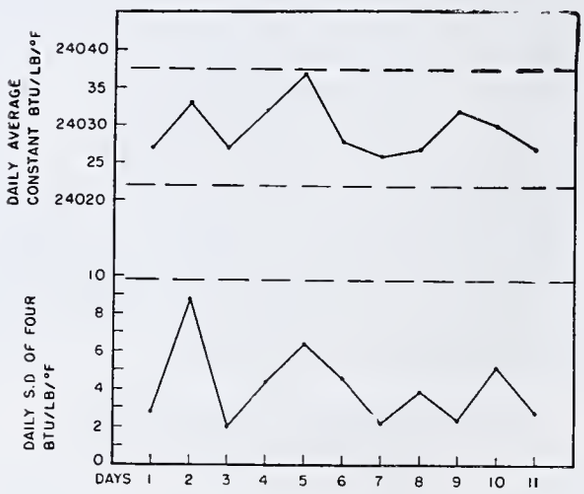

Figure 9. Control Chart for a Calorimeter Constant

The control limits for averaged corrections have been drawn about zero as the mean, in the upper graph, because all corrections would be zero if the burets were perfectly marked. There is more variation among the corrections than corresponds, statistically, to the average range of three consecutive individual calibrations. Therefore, the corrections are applied if they are greater than ±0.01 ml. but no correction is justified if it is ±0.01 ml. or less. In other words, corrections which fall within the control limits based on the variability of the method of calibration are not significant and no gain in accuracy results from applying them.

IMPORTANCE OF PROPER GROUPING OF TEST RESULTS

The manner in which test results are grouped is very important, and the next two control charts illustrate this very well. Figure 8 shows the results of tests on the reproducibility of a calorimeter over a period of about 30 days (*3*). The graph for differences between consecutive days is satisfactory, and the daily individuals show about the same variability as that corresponding to the average difference between consecutive days. The results are outside control limits on two days and there are too many results approaching the control limits. This indicates that there may be more variation from day to day than corresponds to the variation in any one day and the data, as collected, will never show it up.

Figure 9 shows a chart for the calibration of a fuel calorimeter, in which replicate calibrations were made on the same day. Daily standard deviations and averages are again all within their control limits, which, in this case, means that there is no more variation from day to day than corresponds, statistically, to the average variation on any one day. The two charts shown in Figures 8 and 9 illustrate how the same type of experimental work can be made to have different operational meanings merely by carefully choosing the method of grouping the tests.

Another illustration of the importance of picking the proper arbitrary small group is shown in Figure 10. A controlled sample of sodium hydroxide in potassium hydroxide was submitted for routine analysis twice a month for 18 months. The authors (*21*) treated the entire set of results as a single group and concluded that the test method was satisfactory. However when the data are plotted, it seems evident that the monthly range and the individual determinations themselves show less variability with time. If the data are divided in the middle, two periods are obtained during which there is no more variation from month to month than corresponds, statistically, to the average monthly differences; but the limits are reduced approximately one half during the second period. It is possible that some change in the test procedure was made early in 1942 which will account for these improved results. In any event, had a chart such as this been in use prior to August, 1942, the authors might have found an assignable cause for the two low results of that month.

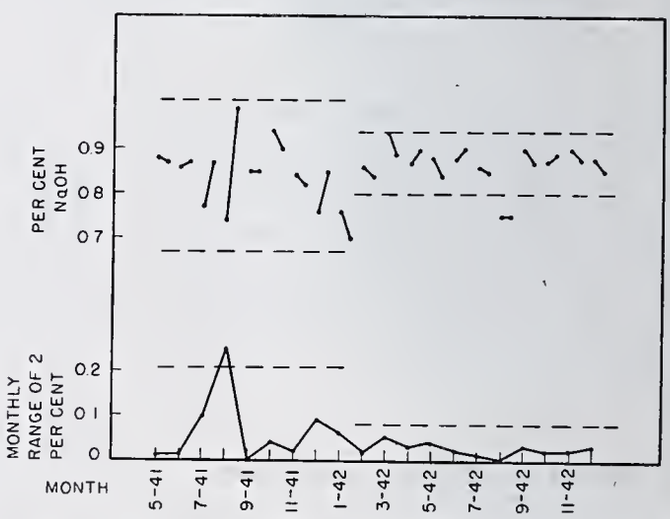

Figure 10. Control Chart for Determination of Sodium Hydroxide in a Potassium Hydroxide Control Sample

Data of Williams and Haines (*21*)

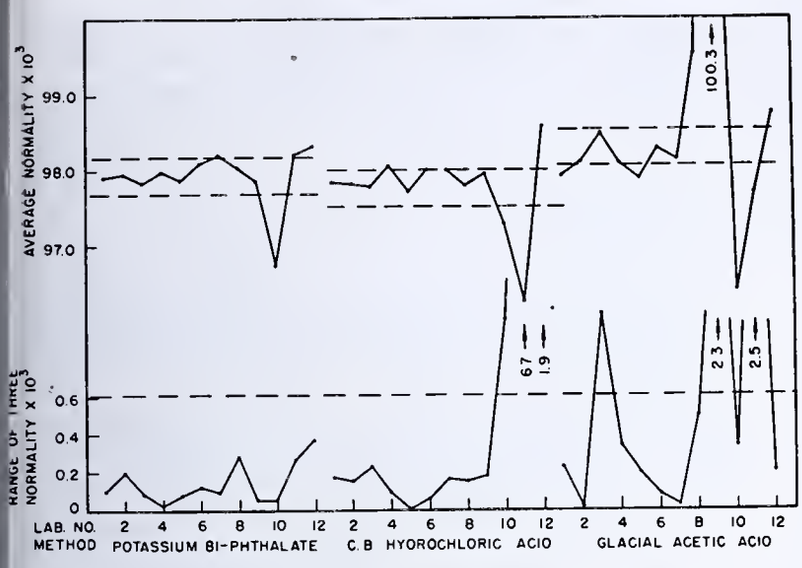

Figure 11. Control Chart for Sodium Hydroxide Standardizations

The application of control chart methods to data already collected often shows that the same amount of experimental work would have yielded a great deal more useful information if it had been planned in a slightly different way.

COMPARISON OF COLLABORATIVE STUDIES OF TEST METHODS

An obvious application of control charts is to the comparison of the results of the collaborative study of test methods, and several examples will be presented to show this. Figure 11 shows the results of such a study of the standardization of 0.1 *N* solutions of sodium hydroxide (*11*). Unknown samples of the solution were standardized in twelve different laboratories by means of three primary standards. The lower graph shows the range of three individual standardizations made at the same time, and the upper graph shows the average of the three. This control chart indicates that there is some uncontrolled factor which affects the ranges of some of the analysts more than corresponds to chance alone, and there is also more variation among the analysts than corresponds, statistically, to the average range of the individual standardizations made by each analyst. Three laboratories are consistently out of control and one laboratory gave bad results when using one of the standards. The cause of these abnormal variations might be in the standardizing procedure, in the controlled sample of sodium hydroxide, in the standardizing materials, or in the analysts, and the control chart should help materially in locating the cause. No conclusions about the relative merits of the three primary standards can be safely drawn until the cause of this lack of control is removed.

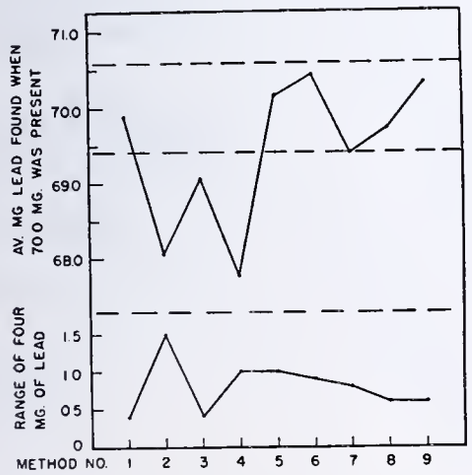

Figure 12. Control Chart for Comparing Various Test Methods for Lead

Data of Lykken (*9*)

The control chart in Figure 12 summarizes data for nine methods of determining lead (*9*). Known solutions containing exactly 70.0 mg. of lead were tested by each method with four replicates at the same time in each test. The methods were all different, five gravimetric and four volumetric. The lower graph shows that the range fluctuations are no greater than correspond to chance alone, although the authors expressed the opinion, based on other considerations, that method 5 showed excessive variation. The control limits for averages were drawn about 70.0 mg. rather than the grand average of all the results because the controlled samples all contained 70.0 mg. of lead. Three of the methods gave excessively low results, based on the average range of the nine tests.

The control chart in Figure 13 summarizes the results of a collaborative study of gravimetric and volumetric methods for determining sulfur in coal (*18*). The sulfur was determined as barium sulfate in both gravimetric methods, but in one case calorimeter bomb washings were used while in the other separate samples of coal were carried through the Eschka method. Bomb washings were used for both volumetric methods but different indicators served to detect the end point of the final sulfate titration with barium chloride.

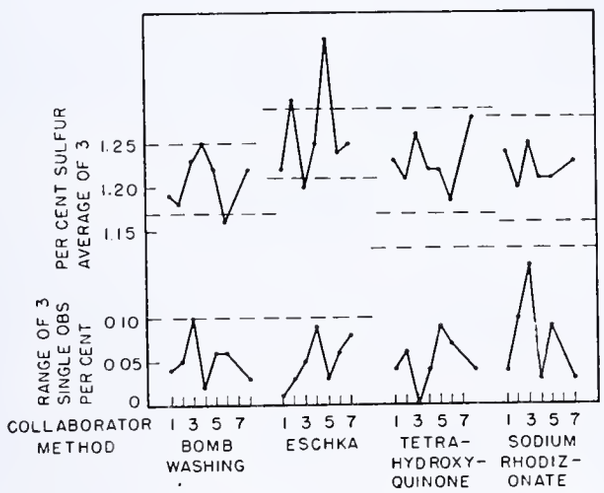

Figure 13. Control Chart for Methods of Determining Sulfur in Coal

Data of Tomkins (*18*)

The lower graph for range of each collaborator's individual determinations shows that the gravimetric methods are appreciably less variable than the volumetric methods and that in both cases the ranges do not show more variation than corresponds to chance alone. One might conclude from this that the volumetric methods are less satisfactory than the gravimetric methods. This is not the case, however, because the upper graph for averages shows that the collaborators cannot agree as well using the gravimetric methods as they do using the volumetric methods.

Unless the cause of this lack of agreement between collaborators using the gravimetric methods can be found and removed it would be better to use the volumetric methods even though they are somewhat less precise. Nothing can be said about the accuracy of the methods because the sulfur content of the controlled samples of coal was not known.

The control chart in Figure 14 shows the results of a collaborative study of three methods for determining the total solids in fish (*19*). Two controlled samples of fish (canned salmon and fresh herring) were tested by five analysts, using a vacuum oven method, a double oven method, in which the samples were heated first to 70° C. and then to 130° C., and a vacuum desiccator method. The weighed samples were dried for arbitrary lengths of time and weighed. The samples were then dried for one additional hour and weighed again.

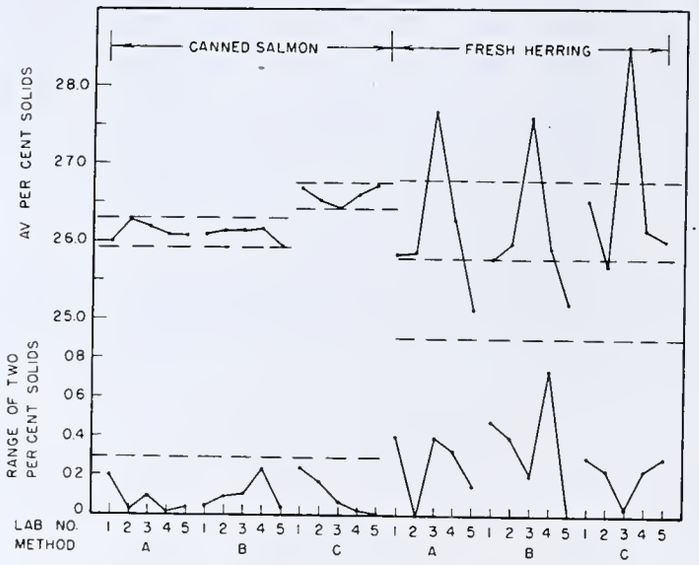

Figure 14. Control Chart for Total Solids in Fish

Data of Tubis (*19*)

The lower graph shows that the variability of differences between duplicate weighings one hour apart is no greater than corresponds to chance for either sample of fish. However, the averages of the five analysts are in much better control for the tests on canned salmon than for the tests on fresh herring, even though the limits for fresh herring are three times as wide. A possible explanation for this difference is that the cooked fish has been broken down so that it requires less drying time than the fresh fish. It seems possible that the results for fresh fish might show satisfactory control if the drying treatment were extended before the first weighing was made.

CONCLUSIONS

Control charts have been found useful in the testing laboratory whenever it is desirable to compare the over-all variability of test data with the average variability of small groups of the data, and they are simpler to understand than the more complicated statistical methods of analysis of variance. Operational meaning can be put into the charts, depending entirely on the manner in which the arbitrary small groups are chosen.

When the small groups consist of replicate tests, made at the same time on the same sample, the resulting control chart subjects the data to the most rigid test that is possible, because most of the uncontrolled factors, which contribute to variations in any test method, affect the groups more than they affect the individual tests within each group.

Test methods which show evidence of control, based on this kind of grouping, may still lack satisfactory precision. In such cases, it is obviously necessary to control more of the factors which contribute to individual test variations within the small groups.

Test methods which show lack of control, based on this kind of grouping, are being influenced by uncontrolled factors which have an unequal effect upon the groups. Often these factors can be found and controlled, but sometimes it is not desirable or, as "Student" pointed out (*17*), even possible to remove them. In such cases, the arbitrary small groups should be changed so that each group will include the effects of the uncontrolled factors.

The control chart for viscosity (Figure 4) illustrates this very well, for if the weekly groups in this chart were changed to groups of replicates tested on the same day, the resulting control limits would be reduced so much that lack of control would be indicated. It is recognized that there is more variation in this test from day to day than that found, on the average, in any one day, and this day-to-day variation is included when the tests are grouped by weeks.

Control chart analysis often shows that routine test methods are not so precise as they were previously thought to be. It also usually shows that the test variations are smaller than the variations in the process which the test is designed to help control. As long as the test variations are no greater than the process variation, replicate testing of single samples may sometimes be uneconomical because the additional evidence of the replicates does not materially help in controlling the process. In such cases, it may be better to replicate the samples in such a manner that more control of the process results, and then test each sample only once.

Only a few of the many applications of control charts have been presented here, but it is safe to say that their use in the analytical laboratory is limited only by the ingenuity of the analyst.

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Determination of Basic Nitrogen in Hydrocarbon Feed Stocks

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mmonia is not retained when very dilute gaseous concentrations : bubbled through dilute sulfuric acid. Storage of such dilute seous mixtures in iron cylinders results in loss of appreciable basic rogen content. Ammonia and amines in concentrations be- een 0.000015 and 0.008% calculated as ammonia have been ccessfully determined in normally gaseous hydrocarbon feed stocks a new method that involves passage of a cooled (about −60° C.) drocarbon sample into a substantially nonaqueous standard solu- n of sulfuric acid, evaporation of the hydrocarbon, and titration the excess acid with standard alkali. About 4 hours are required r a 400-gram sample.

RACE impurities in hydrocarbon gases have a serious and harmful effect upon certain catalytic processes. The mination of such impurities and control of their concentration e dependent upon suitable analytical methods.

Methods for determining minute quantities of water (*1*) d oxygen (*2*) in such gases have been described and the pres- t paper describes a suitable method for ammonia or other sic nitrogen compounds.

In the investigation of methods for determining basic nitrogen, o general types of procedure were considered for transferring e basic compounds from the hydrocarbon to the aqueous edia in which they subsequently could be determined by con- ntional methods. Both methods involved aqueous acid ex- action; in one the sample was treated in the gaseous phase and the other, in naphtha solution.

Where the sample was treated in the gaseous phase, fritted- ass scrubbers containing dilute sulfuric acid failed to retain nall concentrations of ammonia. In a run on 70 grams of hy- rocarbon sample, in which six such scrubbers in series were sed, the first showed 0.00005% of ammonia and the sixth 00004%. In almost every test the second scrubber collected ractically as much base as the first in the series.

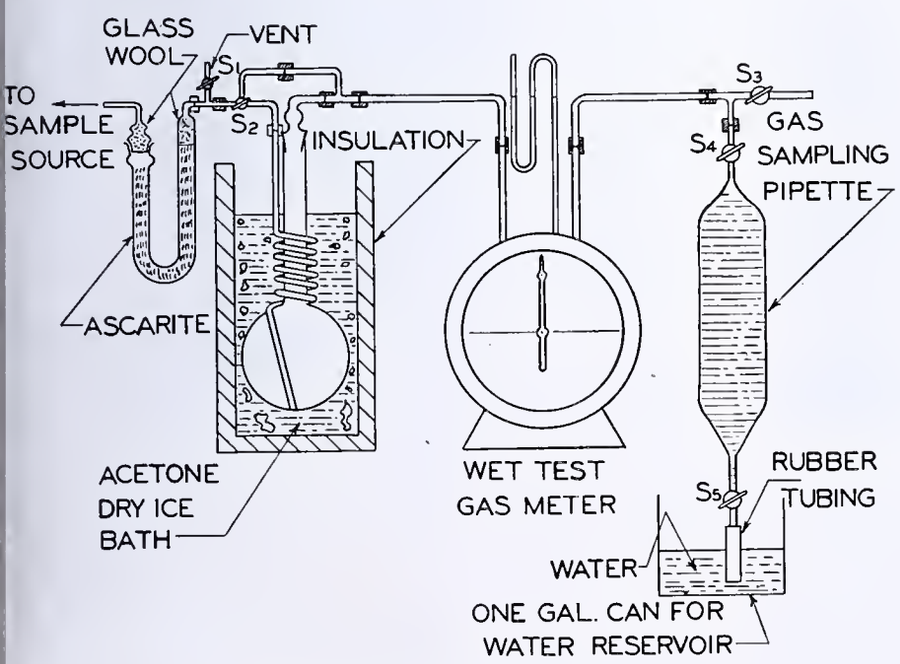

Figure 1. Sample Collection Unit

Treatment of a sample in naphtha solution involved diluting a liquefied sample with naphtha, from which basic compounds were extracted by dilute sulfuric acid using ordinary separatory funnels. Good results were obtained on C_4 hydrocarbon samples but when appreciable quantities of C_3 or lower boiling hydrocarbons were present the manipulations were hazardous and impractical because of the pressures involved.

The procedure which proved satisfactory involves condensation of the hydrocarbon sample into a substantially nonaqueous standard solution of sulfuric acid in acetone, evaporation of the hydrocarbon, and titration of the excess acid to methyl red indicator. The condensation is effected by a dry ice–acetone bath (at about −60° C.); constituents not retained at this temperature are measured on emergence from the condensation flask. Though the lower boiling hydrocarbons are not completely condensed, their basic constituents are quantitatively retained in the nonaqueous acid through which they pass. Sample size is determined from the sum of the condensed and uncondensed portions of the sample, the former by weighing, the latter by gas metering and gravity determination. The time required to complete a determination on a 400-gram sample is not more than 4 hours.

DETAILS OF METHOD

REAGENTS. Sodium hydroxide, aqueous, 0.02 *N*. Prepare by diluting clarified 50% sodium hydroxide solution with carbon dioxide–free water and protecting from carbon dioxide with a soda-lime tube.

Sulfuric acid, 0.02 *N*. Prepare by diluting aqueous 4 *N* sulfuric acid with acetone.

Methyl red indicator, a saturated solution of the free acid in 0.1 *N* aqueous sodium hydroxide.

PROCEDURE. Half fill the condensing bath with acetone and in it carefully immerse a perforated can containing small pieces of dry ice. Acetone will enter the can through its perforated bottom and rapidly dissipate the dry ice. Remove the empty can, refill with dry ice, and again immerse in the acetone bath, repeating the operations until the effervescence, which is violent at first, practically ceases. Then add several pieces of dry ice directly to the bath to maintain its temperature at about −60° C.

Pipet 20 ml. of 0.02 *N* solution of sulfuric acid in acetone into the dry condensing flask and a like portion into a 250-ml. Erlenmeyer flask containing 50 ml. of water; the latter serving as blank is reserved until the sample is titrated, when it also is titrated.

Stopper the condensing flask with the one-hole glass plug and carefully dip it into the cold acetone bath until all the coils of the flask are immersed. Remove the flask from the bath, quickly wipe the bottom, set upon a cork ring on a pan balance, weigh rapidly within 1 gram, and record as tare weight.

Reimmerse the flask in the cold bath and assemble the apparatus as shown in Figure 1. The assembly may be mounted on a suitable rack for convenience in carrying to and from sampling points.

Completely fill the gas-sampling pipet with water, as well as the lower arm of the tee to which it is attached. This may be conveniently accomplished by upward flow. Close stopcock S_4 and leave stopcock S_5 open to the water reservoir.

Introduce the sample through the Ascarite scrubber, and by manipulating stopcock S_2 bypass the condensing flask for a few moments to flush the tubing through stopcock S_3. A rate of

passage as high as 170 liters (6 cubic feet) per hour as indicated by the gas meter has been found to yield good results. Open vent S_1, and record the initial reading of the meter, its temperature, and the barometric pressure. Readjust stopcock S_2, close S_1, and start introduction of the sample into the flask. Add pieces of dry ice as required to the condensing bath to maintain its temperature at about −60° C. If the sample is completely retained in the condensing flask, the meter will, of course, indicate no gas passage. If uncondensed gases are present, take a sample for gravity determination after about 0.2 cu. foot (5663 cc.) have by-passed the sampling pipet and assured purging of air, etc. For a truly representative sample, small portions should be drawn over the entire collecting period after purging, but for most purposes the sample may all be collected at one time. Regulate stopcocks S_3 and S_4, so that substantially atmospheric pressure is indicated on the manometer with which the gas meter is equipped. When approximately 1 liter of gas has been collected, detach sampling pipet and determine the specific gravity of the gas against air.

When sufficient sample has been taken, stop its flow, detach the condensing flask, and weigh rapidly as before. In most cases a 400-gram sample is adequate, but when extremely low concentrations are involved, the sample should be increased. Record the new weight and meter reading.

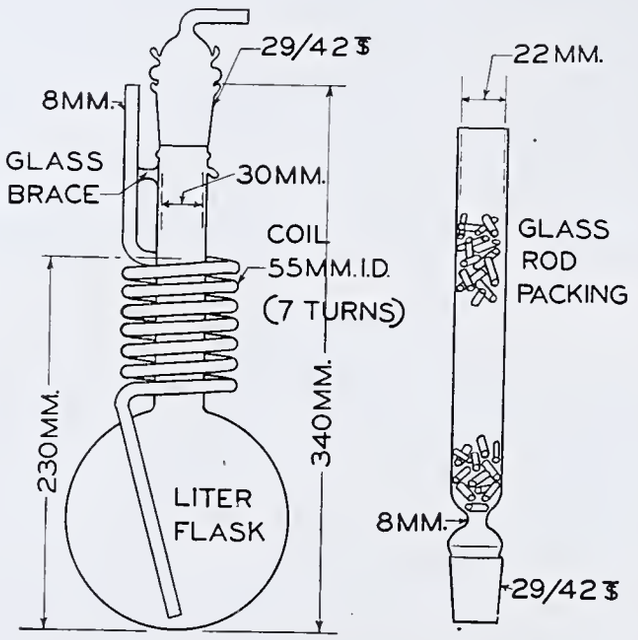

Figure 2. Glass Condensing Flask-Evaporation Trap

Gently swirl the flask and set it on a cork ring in a good hood for evaporation of its hydrocarbons. Remove the one-hole plug from its neck. Pour water through the evaporation trap shown in Figure 2, drain a few moments, and insert in the neck of the flask. Close the inlet tube of the condensing flask with a short piece of rubber tubing fitted with a glass plug to prevent ejecting liquid during the subsequent evaporation step.

To hasten evaporation carefully immerse the flask in a bath of water at about 60° C. Less than 45 minutes are required to evaporate the hydrocarbons. Upon evaporation of the sample, add 50 ml. of water to the flask through the trap. Remove the trap as well as the rubber tubing and plug from the inlet tube. Rinse down the coil or inlet tube of the flask with water, add methyl red indicator to the flask, and titrate with standard 0.02 N sodium hydroxide. Titrate the blank in a like manner.

CALCULATIONS. *Noncondensed Gas.* (The calculation for noncondensed gas is generally sufficient. However, to be strictly correct, the volume of noncondensed gas, V, and its specific gravity, G, should be calculated to a dry basis.)

$$\text{Noncondensed gas (grams)} = \frac{V \times 28.32 \times G \times 1.223 \times 288.6 \times P}{T \times 760}$$

where V = cubic feet measured at meter
G = specific gravity of noncondensed gas (specific gravity of air = 1.0000)
T = average meter temperature, degrees absolute
P = barometric pressure, mm. of mercury
28.32 = liters per cubic foot
1.223 = weight in grams of 1 liter of air at 15.6° C. and 76 mm. of mercury

This may be simplified to:

$$\text{Noncondensed gas (grams)} = \frac{13.15 \times V \times G \times P}{T}$$

Per Cent Basic Nitrogen.

$$\%\ \text{basic nitrogen (calcd. as } NH_3) = \frac{(A - B) \times N \times 1.7}{\text{grams of sample}}$$

where A = ml. of sodium hydroxide required to titrate the blank
B = ml. of sodium hydroxide required to titrate the sample
N = normality of sodium hydroxide
Grams of sample = grams of noncondensed gas plus grams of condensed gas

DISCUSSION

The accuracy of this method is indicated by the typical values shown in Table I. Concentrations of ammonia or amine as low as 0.000015% and as high as 0.008%, calculated as ammonia, have been successfully determined. It is apparent that as the concentration decreases to a minimum, a small titration error will contribute to a large percentage error.

Ammonia, methylamine, dimethylamine, trimethylamine, ethylamine, isopropylamine, and methylethylamine, ranging in boiling points from −33° to +35° C., were all used to prepare known samples for analysis. The quality of the results was unaffected by the nature of the base employed.

In preparing the samples, ammonia and methylamine were measured gasometrically in small sample pipets under controlled conditions of temperature and pressure, and then flushed out of the measuring pipets with the hydrocarbon gas concerned.

Samples containing the other nitrogen bases were prepared by adding standard alcoholic or pentane solutions of the base to liquefied hydrocarbons.

Acetone is used to dilute the aqueous 4 N sulfuric acid. The initial dilution is made with water because it is expected that

Table I. Typical Data Obtained with This Method

Blending Gas[a]	Base Taken	Base Calculated as Ammonia, % Taken	Found
A	Ammonia	0.00123	0.00123
B	Ammonia	0.00022	0.00022
B	Ammonia	0.00127	0.00129
C	Ammonia	0.00125	0.00127
C	Methylamine	0.00097	0.00097
D	Methylamine	0.00147	0.00147
E	Methylamine	0.000016	0.000024
E	Methylamine	0.00120	0.00120
E	Ethylamine	0.000137	0.000137
F	Ammonia	0.00073	0.00074
F	Ammonia	0.00004	0.00004
G	Ammonia	0.00003	0.00004
G	Ammonia	0.00223	0.00227

[a] Composition of blending gases shown in Table II.

Table II. Composition of Blending Gases

	Methane	Ethane	Propane	Iso-butane	*n*-Butane	Heavier
			Per cent by volume			
A	...	...	100	...	...	...
B	12.4	37.1	49.0	...	1.5	...
C	...	8.4	44.5	0.5	45.8	0.8
D	...	14.6	41.0	1.9	42.5	...
E	...	...	...	...	100	...

	Ethylene	Propylene	*n*-Butane	Iso-butane	1-Butylene Iso-butylene	Iso-pentane	1,3-Butadiene
F	1.6	3.3	...	69.7	22.9	2.5	...
G	...	...	53.5	...	...	...	46.5

neutralization reactions will proceed with greatest ease in the presence of water. Acetone is used, because it is a solvent for both liquefied hydrocarbons and water, apparently has no effect upon the standard acid, and lowers the freezing point of the solution to below −60° C. The titer of 0.02 *N* sulfuric acid in acetone dropped less than 1% in 144 hours. Other solvents, ketones and alcohols, were not so satisfactory for one or more reasons involving high freezing point, poor miscibility, or instability of the titer of the standard acid prepared with them.

It was found that samples cannot be kept or weighed in iron containers, though free of brass valves and fittings, since reaction or adsorption occurs, reducing the base content of the hydrocarbon. Solutions of ammonia in *n*-butane, kept in iron cylinders, changed from an initially determined value of 0.00081% to lower and lower values until after 16 hours' storage only 0.0001% remained. Solutions of methylamine in *n*-butane, similarly stored, changed from 0.00149 to 0.00103% in 16 hours. Similar solutions prepared with these bases, as well as with bases up to and including isopropylamine, but in pressure glass tubing, retained their initial concentrations after storage, demonstrating that loss of base was not due to reaction between it and the hydrocarbon solvent. The Florence flask, equipped with condensing coil, eliminates the need for metal sample containers.

ACKNOWLEDGMENT

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Use of High-Frequency Oscillators in Titrations and Analyses

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Energy absorption from high-frequency fields forms the basis for a new method of conductometric analyses. Distinct advantages are that this method eliminates the use of electrodes, and that values may be read directly on a meter. It is very flexible and highly sensitive. Any changes in ionic or dipole content in ionized or un-ionized solutions may be followed. The electrical circuit is described and a variety of types of titrations are presented.

ELECTRICAL methods for titrations and analyses are generally limited in their scope and application by the necessity of using electrodes of various types. Galvanic methods usually are restricted to ionized solutions and ordinarily are not usable in organic liquids, particularly if the organic liquid is a very poor ionizing medium. Conductometric methods have a wide field of application but frequent readjustments are usually required. By using the field of a high-frequency oscillator it is possible to produce ionic or dipole motion without the introduction of electrodes. The energy required to produce this motion causes a change in the oscillator current which is easily measured. Thus when an oscillator is loaded by the introduction of a liquid or a solution into its tank circuit, its characteristics are altered. Chiefly three factors govern the magnitude of the loading: the type and circuit constants of the oscillator, the volume of the solution and its location within the tank circuit, and the conductance of the solution due to its ionic or dipole concentration. The changes in conductance during a reaction cause variations in loading, whereby the course of the reaction can be followed.

Oscillators differ widely in the manner in which they load. In Figure 1 are shown types of loading curves which can be obtained from various oscillators or even from the same oscillator by changing its frequency or its circuit constants. Conditions which produce linear curves of steep slope are desirable, since oscillators producing such curves show high and uniform sensitivity throughout their loading range. However, oscillators with such high sensitivity also load out of oscillation readily. Therefore provision for restoring oscillation is highly essential. A study of the characteristics of various types of oscillators (*5*) indicated that the tuned-plate tuned-grid oscillator was the most adaptable to analytical work, since it could be made highly sensitive and yet maintain reasonable stability, and could be readily brought back into oscillation when necessary.

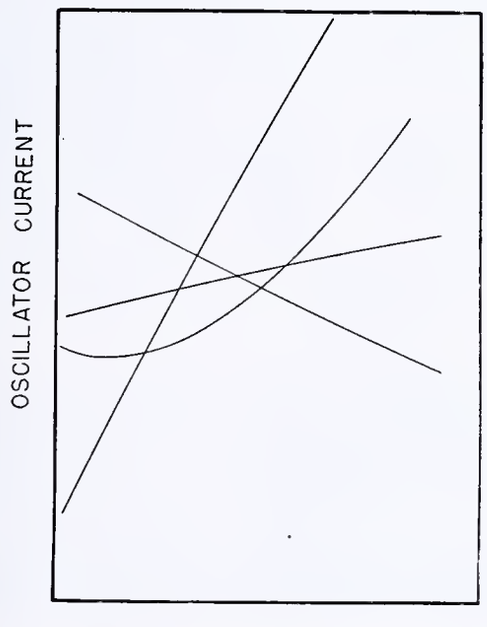

Figure 1. Typical Loading Curves

ELECTRICAL CIRCUIT

In Figure 2 is shown the electrical circuit of a tuned-plate tuned-grid oscillator with a sensitive metering system in the positive power-supply lead. A regulated power source furnishes high voltage. Oscillations occur when the two tank circuits, L_1C_1 and L_2C_2, are resonant at approximately the same frequency. The condenser, C_1, is the primary factor in determining the frequency of oscillation, while condenser C_2 controls the excitation to the grid of tube A. Any triode vacuum tube having good oscillating characteristics may be used. Current flowing through resistance R_1 produces the grid-bias voltage. The trimmer condenser, C_3, by-passes high-frequency energy around R_1 and is adjusted to give maximum sensitivity to loading in the plate tank circuit. Introduction of the glass tube, T, containing the solution into coil L_1 accomplishes the loading. Condensers C_4 to C_9, inclusive, and choke coil L_2 reduce stray high-frequency

currents and eliminate body-capacity effects, thus stabilizing the circuit.

The oscillator current is read on milliammeter M_3, which also assists in determining the manner in which the circuit goes in or out of oscillation. By adjustment of resistance R_4 in the series R_3-R_4-R_5, the voltage across resistance R_2 can be balanced by an equal and opposite voltage across R_3 and a portion of R_4.

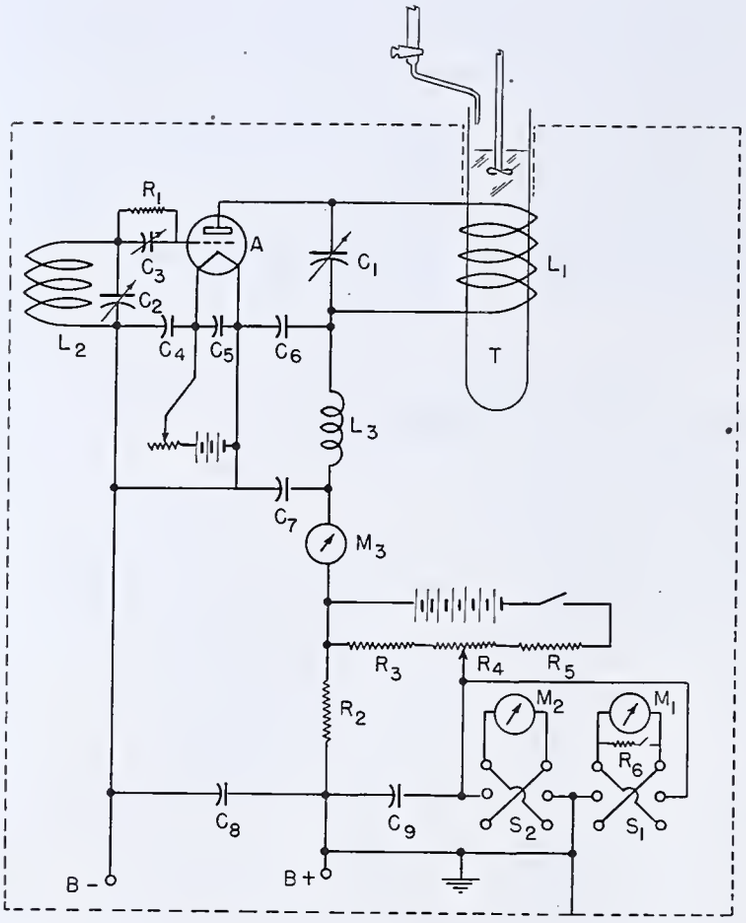

Figure 2. Electrical Circuit

With the protective shunt, R_6, in the circuit and with switch S_1 closed, the microammeter, M_1, shows the approximate state of balance. Closer adjustment of R_4 then permits removal of R_6 from the circuit. Reversing switches S_1 and S_2 serves to increase the range of microammeters M_1 and M_2 without reducing sensitivity. Thus, if M_1 and M_2 have ranges of 0 to 200 and 0 to 50 microamperes, it is possible to read over a range of 400 microamperes without resetting, and still maintain an accuracy in reading of 0.2 microampere. The values of the various circuit constants are not highly critical. The following will give good results.

R_1. Resistance, 40,000 ohms
R_2. Resistance, 1,000 ohms
R_3, R_5. Resistance, 10,000 ohms
R_4. Wire-wound potentiometer, 10,000 ohms
C_1, C_2. Variable condenser, 100 micromicrofarads
C_3. Adjustable mica condenser, 100 micromicrofarads
C_4–C_9. Mica condenser, 0.01 microfarad
S_1, S_2. Double-pole double-throw anticapacity switch
M_1. Microammeter, 0 to 200 microamperes
M_2. Microammeter, 0 to 50 microamperes
M_3. Milliammeter, 0 to 150 milliamperes
L_1, L_2. Coil, 4 turns of No. 6 copper wire, spaced 1.5 inches; coil diameter, 2.25 inches
L_3. Radio frequency choke, 2.5 millihenries
A. Vacuum tube, type 801

High sensitivity is obtainable by increasing the size of R_2 up to certain limits, or by adjusting C_2 and C_3 to such values that tuning with C_1 causes the circuit to go in or out of oscillation with extreme rapidity. As sensitivity is increased a slow drift in the meter reading frequently becomes apparent. Good shielding and grounding are essential. The shield around T fits snugly and extends well below the liquid level inside the tube. The length of this shield is determined experimentally and is adjusted so that changes in the liquid volume during analysis will have no effect upon the loading.

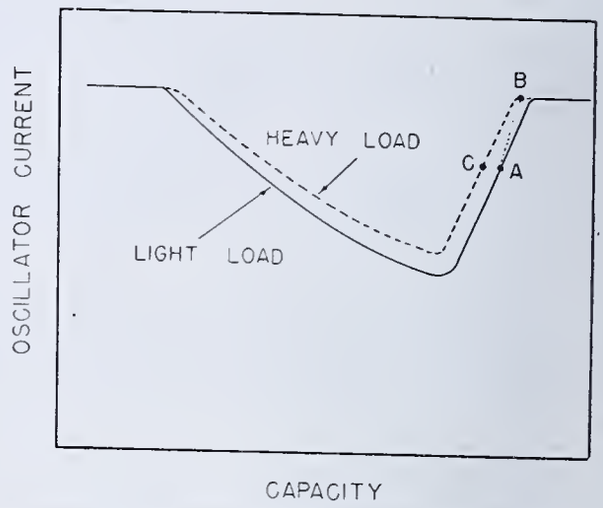

Figure 3. Variation of Oscillator Current with Tuning of C_1

The choice of frequency is not critical, except that an oscillator of a given design may have maximum sensitivity at a certain frequency. Frequencies in the range of 15 to 20 megacycles give satisfactory results. At higher frequencies the control of stray currents is more difficult, while coils and condensers tend to become excessively large at lower frequencies. Figure 3 illustrates the manner in which a tuned-plate tuned-grid oscillator responds to tuning by C_1. The circuit is in oscillation only when the two tank circuits are resonant at approximately the same frequency. Increase in loading during titration causes a change in the impedance and in the resonant frequency of the plate tank circuit. However, its original resonant frequency can be restored by changing the capacitance in the plate tank circuit. Thus, with C_1 set to give good loading characteristics as at A, a sufficient

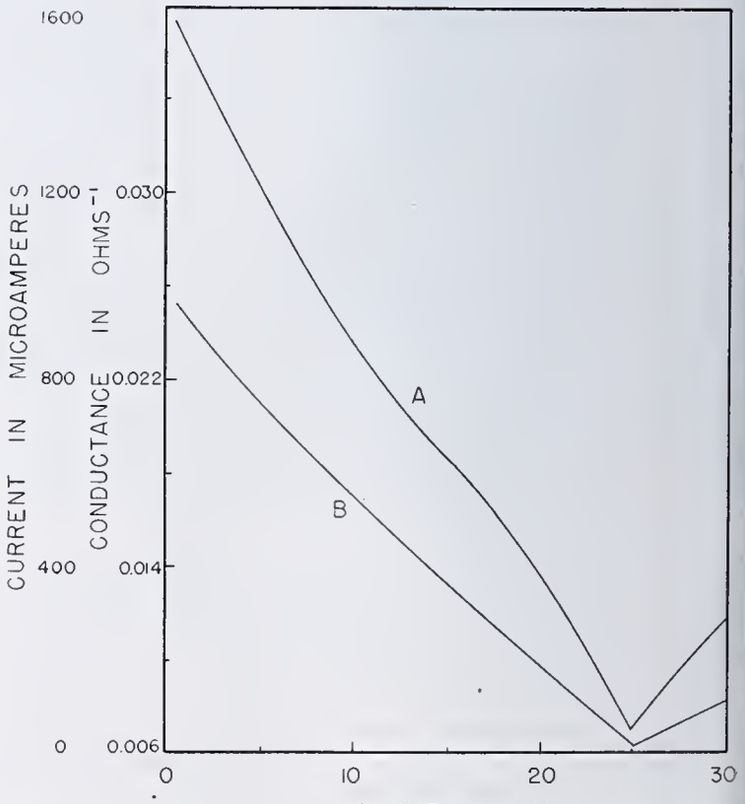

Figure 4. Titration of Hydrochloric Acid with Sodium Hydroxide

A. High-frequency method. *B*. Conductance method

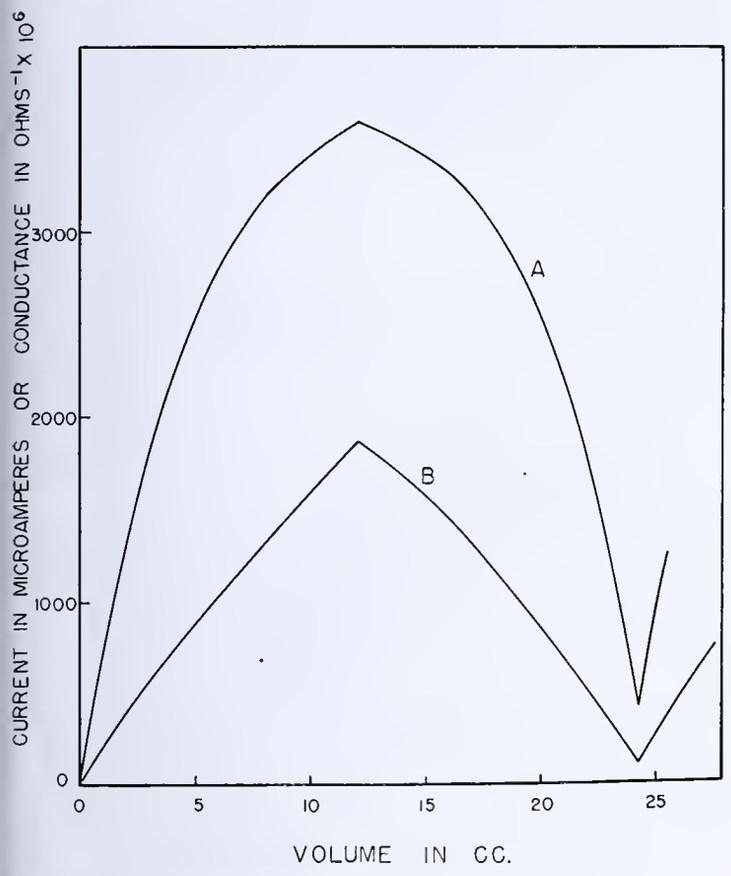

Figure 5. Titration of o-Phthalic Acid in Acetone with Sodium Methylate in Methanol

A. **High-frequency method.** ***B.*** **Conductance method**

increase in loading during titration may cause the circuit to go out of oscillation as at B. Then by simply retuning with C_1, the circuit can readily be brought back into oscillation. Since the two loading curves shown on Figure 3 are nearly parallel up to the point where the circuit begins to go out of oscillation, the slopes of the titration curves are not materially changed by retuning with C_1 in this manner, particularly if the retuning is done before the circuit goes out of oscillation. In actual practice C_1 can be very conveniently used as a means of resetting when the current increases or decreases beyond the range of the microammeters. Minor adjustments can be made with R_4. With this technique no observable changes in the slopes of the titration curves are produced by resetting.

TYPICAL ANALYSES

In carrying out a titration the solution in tube T is suspended within coil L_1 and the motor stirrer is started. By means of C_1, and also C_3 if necessary, the circuit is adjusted so that it goes into and out of oscillation with the desired sensitivity as observed on M_3. Balance on meters M_1 and M_2 is obtained by adjustment of R_4. If the temperature of the solution differs from that of the interior of the system, a slow drift of the meter needle is apparent until thermal equilibrium is reached. Ordinarily this effect can be neglected in titration.

In the various types of titrations, illustrated in Figures 4 to 9, 25 cc. of solution in the order of 0.1 N were diluted with 100 cc. of water and were titrated with solutions also in the order of 0.1 N. End points appear as changes in the slopes of the curves. If the disappearing ion has a higher conductance than the ion replacing it, the excess of the titrating agent beyond the end point produces a reversal in the curve. Comparison is made between this method and the usual conductance method on Figures 4 and 5. The scale for the microampere ordinate is greatly reduced for comparison purposes. In cases such as the titration of acidified ferrous ammonium sulfate solution with potassium permanganate solution, addition of the titrating agent beyond the end point actually dilutes the solution and lowers its conductance, since the solution contains a relatively high concentration of sulfuric acid. Thus as seen from curve A on Figure 8, the end point appears but no reversal takes place. If, however, the conductance of the potassium permanganate solution is increased by the addition of inert potassium sulfate, the typical reversal occurs at the end point as shown by curve B. Jander and his co-workers (*1*, *3*, *4*), in their work on conductometric titrations in solutions of high foreign salt content, add the concentrated titrating agent from a specially constructed microburet in order to avoid large volume changes. However, the sensitivity of the high-frequency method is sufficiently great, so that adding relatively large amounts of inert salt with the titrating agent does not destroy its accuracy.

Titrations in organic solvents or mixtures of organic solvents frequently present interesting problems. Since the degree of ionization is usually low, loading due to rotation of dipoles may constitute an appreciable part of the total effect. Furthermore, if the titration involves more than one solvent, changes in the

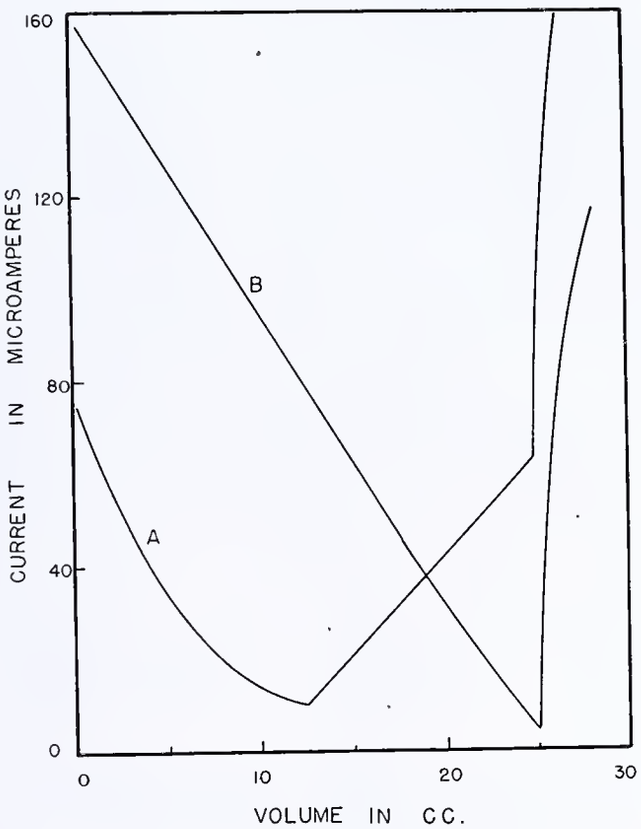

Figure 6. Titration of Sodium Carbonate with Hydrochloric Acid (*A*) and of Potassium Chloride with Silver Nitrate (*B*)

degree of ionization and in the dipole content during titration materially affect the slope of the curve. The end point on curve B, Figure 9, is completely masked, owing to the increase in the number of methanol molecules which are more polar than the acetone molecules, and the increase in the solubility and the degree of ionization of the sodium benzoate as methanol progressively enters the solution. When the sodium methylate is made up in benzene with only sufficient methanol to maintain solubility, a sharp end point is obtained as seen by curve C. Initially, conductance increases until the limit of solubility of the sodium benzoate is reached, and thereafter decreases because of the removal of benzoic acid as insoluble sodium benzoate. This suggests a possible method for the determination of solubilities.

Results of these titrations are listed in Table I. The known end point values are those obtained by standard volumetric methods or calculated from the known strengths of the solutions. The observed end point values are obtained by use of the oscillator.

Table I. Comparison of Known and Observed Values

Substance Titrated	Titrating Agent	Known End Point, Cc.	Observed End Point, Cc.
H_3PO_4	NaOH	25.25	12.66
			25.20
			37.87
Na_2CO_3	HCl	12.20	12.18
			24.40
$FeSO_4(NH_4)_2SO_4$	$KMnO_4$	25.00	24.98
$FeSO_4(NH_4)_2SO_4$	$KMnO_4$–K_2SO_4	25.04	25.03
$Na_2C_2O_4$	$KMnO_4$–K_2SO_4	25.13	25.10
KCl	$AgNO_3$	24.92	24.90
C_6H_5COOH in acetone	CH_3ONa in benzene	23.98	24.00
HCl	Na_2CO_3	25.50	25.50
$C_6H_4(COOH)_2$ in acetone	CH_3ONa in MeOH	24.00	12.00 24.00

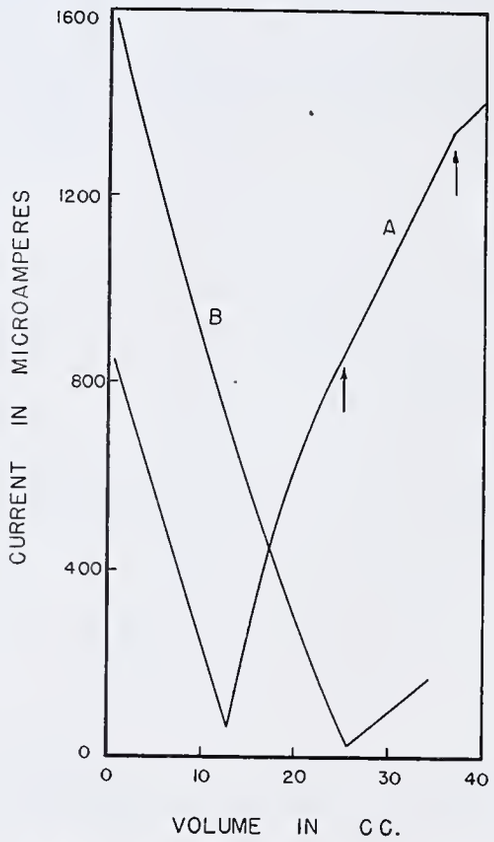

Figure 7. Titration on Phosphoric Acid with Sodium Hydroxide (*A*) and of Hydrochloric Acid with Sodium Carbonate (*B*)

DILUTE SOLUTIONS

Adjustment of C_2 and C_3 and tuning with C_1 can cause the circuit to go in or out of oscillation with such extreme rapidity that even slight changes in conductance produce relatively large changes in the oscillator current. Curve *A*, Figure 10, shows the titration of 25.00 cc. of 0.01000 *N* potassium chloride solution, diluted to 300 cc., with 0.01000 *N* silver nitrate solution. The minimum conductance point at 23.90 cc. does not coincide with the true equivalence point of 25.00 cc. for several reasons. The silver chloride becomes more soluble as the equivalence point is approached and dilution of ions takes place as titration proceeds. However, a simple approximate correction can be made in the following manner:

If x is the number of gram-ions of chloride ion present at any time, then K/x, NV_1, and $NV_1 - (x - K/x)$ are the amounts of silver, potassium, and nitrate ions, respectively, where N is the normality of the titrating agent, and V_1 is the volume of the titrating agent at the equivalence point. K is used as $K_s(V_1 + V_2)^2$ where V_2 is the volume of the solution before titration. The term $(x - K/x)$ represents the amount of chloride ion for which no silver ion has been added. A close approximation of the conductance of the solution at any time prior to the equivalence point is shown by Equation 1.

$$\text{Conductance} = \frac{\lambda_{K^+}NV_1 + \lambda_{Cl^-}\,x + \lambda_{Ag^+}\frac{K}{x} + \lambda_{NO_3^-}\left[NV_1 - \left(x - \frac{K}{x}\right)\right]}{V_1 + V_2 - \frac{x}{N}} \quad (1)$$

If the derivative is taken and equated to zero, the quadratic Equation 2 results.

$$[V_1(\lambda_{K^+} + \lambda_{Cl^-}) + V_2(\lambda_{Cl^-} - \lambda_{NO_3^-})]x^2 + \left[2(\lambda_{Ag^+} + \lambda_{NO_3^-})\frac{K}{N}\right] \times$$

$$x - (V_1 + V_2)(\lambda_{Ag^+} + \lambda_{NO_3^-})K = 0 \quad (2)$$

In the solution of this equation coefficient b is very small in comparison to $\sqrt{-4ac}$ and therefore at minimum conductance:

$$x = \sqrt{-c/a} \quad (3)$$

or

$$x = \sqrt{\frac{(V_1 + V_2)^3(\lambda_{Ag^+} + \lambda_{NO_3^-})K_s}{V_1(\lambda_{K^+} + \lambda_{Cl^-}) + V_2(\lambda_{Cl^-} - \lambda_{NO_3^-})}} \quad (4)$$

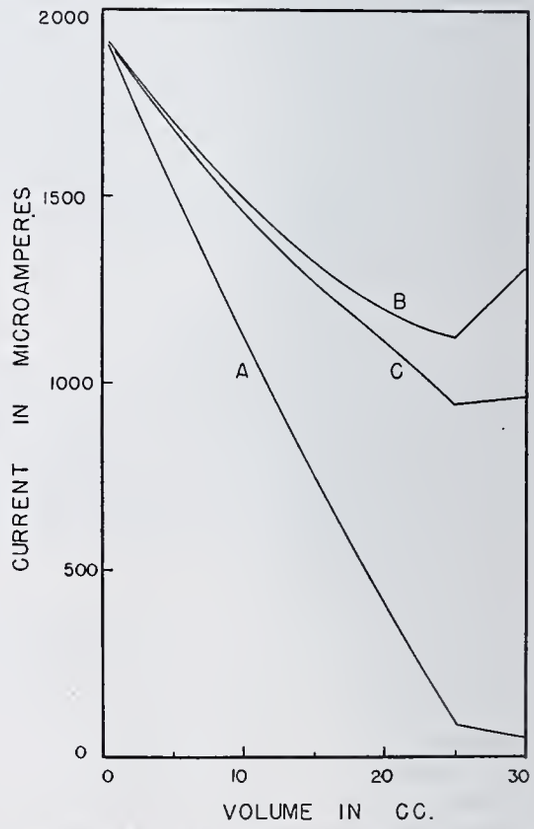

Figure 8. Effect of High Ion Concentration

A. Titration of 0.1 *N* ferrous ammonium sulfate with 0.1 *N* potassium permanganate
B. Titration of 0.1 *N* ferrous ammonium sulfate with 0.1 *N* potassium permanganate and 0.4 *N* potassium sulfate
C. Titration of 0.1 *N* sodium oxalate with 0.1 *N* potassium permanganate and 0.5 *N* potassium sulfate

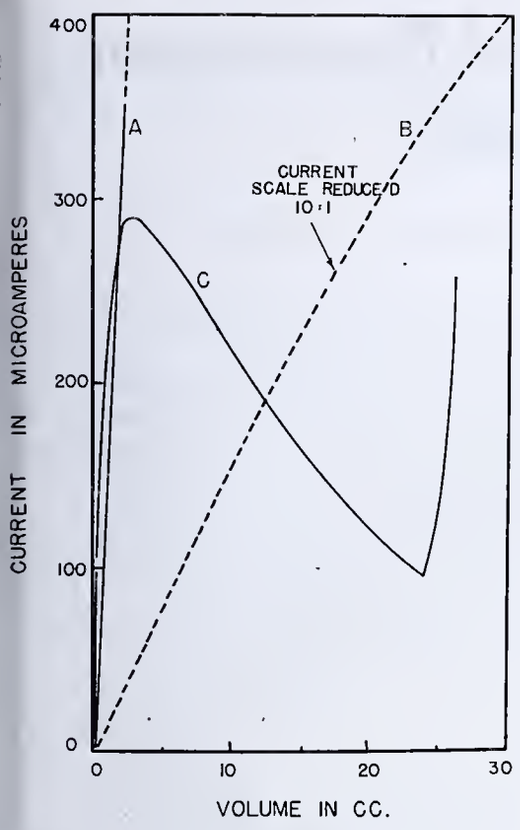

Figure 9. Titration of Benzoic Acid in Acetone

A, B. With sodium methylate in methanol
C. With sodium methylate in benzene

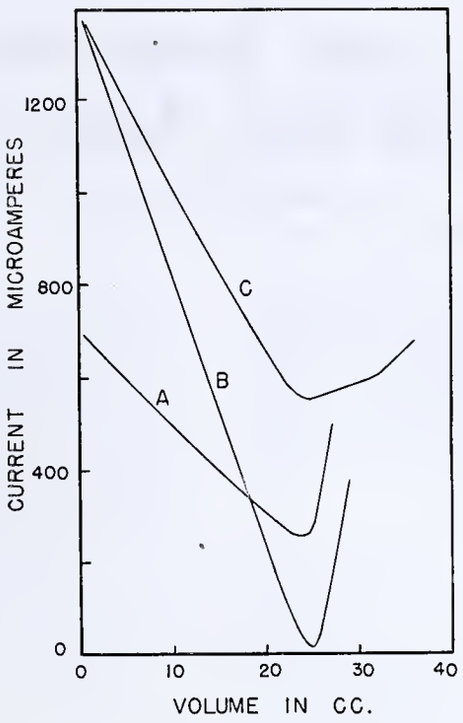

Figure 10. Titrations in Dilute Solutions

A. 0.01 *N* potassium chloride with silver nitrate
B. 0.01 *N* potassium chloride with silver acetate
C. 0.001 *N* hydrochloric acid with sodium hydroxide

Figure 11 illustrates another type of analysis which may be performed readily by this method. The curve shows the variation in oscillator current as the concentration of hydrogen chloride in dry benzene is changed. Since a change in hydrogen chloride concentration from 0 to 0.275% by weight produces a change of 100 microamperes, it is possible to read a change in hydrogen chloride content in the order of 0.0006% by weight.

To some extent temperature changes affect the oscillator current. With an increase in temperature the ionic conductances become greater, causing an increase in the current. The temperature effect on the dielectric constant causes a change in the opposite direction.

In addition to titrations, this simplified conductance method can be applied for following reaction rates, control work, etc. Wherever changes in ionic or dipole conductances occur, they may be readily observed by this means.

MacInnes, Shedlovsky, and Longsworth (*6*) give the ionic conductances at 25° C. of potassium, chloride, silver, and nitrate ions at 73.50, 76.32, 61.90, and 71.42 reciprocal ohms, respectively. The value of K_s for silver chloride is calculated as 1.7×10^{-10} from its solubility (*2*) at 25° C. Using these values in Equation 4 and replacing V_1 by its approximate equivalent of 0.0239 liter (the volume at minimum conductance), the amount of chloride ion at minimum conductance is 1.24×10^{-5} gram-ion. K/x is 0.15×10^{-5} gram-ion and $(x - K/x)$ is 1.09×10^{-5} gram-ion. This is equivalent to 1.09 cc. of 0.01000 *N* silver nitrate solution. Addition of this correction to the 23.90 cc. gives an end point of 24.99 cc.

In Equation 4 the term $(\lambda_{Cl^-} - \lambda_{NO_3^-})$ appearing in the denominator represents the difference in the ionic conductances between the disappearing ion and the ion replacing it. If instead of the nitrate ion, the acetate ion, which has a conductance 40.87 reciprocal ohms (*6*), is used, this difference becomes greater, thereby decreasing the value of x. On curve *B*, Figure 10, the minimum conductance point is much sharper and appears at 24.68 cc. when silver acetate is used. Calculating the correction from Equation 4 but replacing the ionic conductance of the nitrate ion with that of the acetate ion, the end point is found as 25.05 cc.

It can be seen in Equation 4 that small values of K_s, as well as large differences in conductance between the disappearing ion and the ion replacing it, will produce small corrections. In acid-base titrations the hydrogen ion with a conductance of 349.72 reciprocal ohms (*6*) disappears and is replaced by a positive ion of much lower conductance. Also, since the ion product constant is 1.0×10^{-14}, the corrections will become very small or negligible. As shown on curve *C*, Figure 10, the titration of 25.00 cc. of 0.000996 *N* hydrochloric acid solution in 300 cc. of redistilled water with 0.001003 *N* sodium hydroxide solution produces a minimum conductance point at 24.80 cc. The known end point is 24.83 cc. The correction of 0.003 cc. is negligible. The change in the slope of the curve at 32 cc. is due to titration of carbon dioxide which has dissolved during titration. Its position varies with the time used for titration.

Cases may occur in which the disappearing ion has a lower conductance than the replacing ion. Then, since the curve has a positive slope throughout, no minimum point is produced and Equation 4 does not apply. However, the excess of titrating agent entering the solution as the end point is passed causes a break in the curve and no correction is necessary.

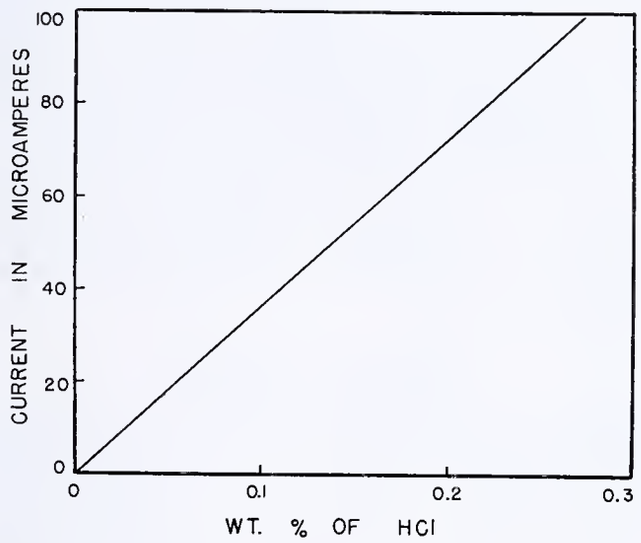

Figure 11. Determination of Hydrogen Chloride in Benzene

ACKNOWLEDGMENT

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Chromatography in the Separation and Determination of the Basic Amino Acids

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The individual basic amino acids were separated chromatographically from amino acid mixtures and protein hydrolyzates by a single adsorption on Lloyd's reagent. Lysine was determined by a modification of the ninhydrin colorimetric reaction, histidine by Pauly's diazo reaction, and arginine by the Sakaguchi reaction. The method is simple, rapid, and reliable.

THE present chemical methods for the determination of the basic amino acids, particularly lysine, are time-consuming and not entirely specific. Previous investigators (*1, 2, 6, 7, 12, 15*) have shown that it is possible to separate chromatographically the basic amino acids from the nonbasic ones and then separate the individual basic amino acids by subsequent adsorption and elution procedures. Very little work was done with protein hydrolyzates, but the results indicated that chromatography could be applied successfully to them.

The purpose of this investigation was to find, if possible, a chromatographic method for complete separation of the basic amino acids, particularly lysine, from known mixtures of amino acids and from protein hydrolyzates. Ideally such a method should require only one adsorption. Simple methods should be used for the determination of lysine, histidine, and arginine after their separation.

In this investigation the individual basic amino acids were separated from known mixtures of amino acids and protein hydrolyzates by a single adsorption on Lloyd's reagent. Lysine was determined by a new modification of the ninhydrin colorimetric reaction, histidine by Pauly's diazo reaction, and arginine by the Sakaguchi reaction.

GENERAL CONSIDERATIONS

Adsorption columns were prepared with Lloyd's reagent and the filter aid, Hyflo Super-Cel, in 19-mm. tubes. Lloyd's reagent was selected because Ackermann and Fuchs (*1*) had shown that it completely adsorbed the basic amino acids. Quantities of 12 to 20 grams of the adsorbent mixture (one-third Lloyd's reagent by weight) were used.

The basic amino acids were adsorbed on the columns from water or from 1% hydrochloric acid solutions of their hydrochlorides. The interchanging of water and 1% hydrochloric acid as solvents had little effect on the adsorption characteristics of the basic amino acids if the amount used for each adsorption were less than 20 ml. Samples of 1 to 8 mg. of lysine and histidine and 2 to 16 mg. of arginine were used for each adsorption. The larger amounts of arginine were used because many proteins contain larger quantities of arginine than of lysine and histidine.

The amino acid mixtures were adsorbed from 1% hydrochloric acid solutions. A representative mixture was: 20 mg. of *dl*-alanine, 25 mg. of *dl*-aspartic acid, 20 mg. of *l* (−)-cystine, 25 mg. of *l* (+)-glutamic acid, 30 mg. of glycine, 20 mg. of *dl*-isoleucine, 20 mg. of *dl*-leucine, 10 mg. of *dl*-methionine, 20 mg. of *dl*-norleucine, 20 mg. of *dl*-phenylalanine, 20 mg. of *dl*-threonine, 20 mg. of *l* (−)-tryptophane, 50 mg. of *l* (−)-tryosine, 20 mg. of *dl*-valine, 8 mg. of *l* (+)-arginine, 4 mg. of *l* (−)-histidine, 4 mg. of *l* (+)-lysine, and 100 mg. of ammonium chloride, per 10 ml. of solution.

QUALITATIVE STUDIES ON AMINO ACID ADSORPTION

Although the adsorption and elution characteristics of many compounds are affected by the presence of similar compounds, the basic amino acids behaved the same under identical conditions of adsorption and elution either alone or in amino acid mixtures. Thus, when conditions for a separation method for the basic amino acids were established, tests for the beginning and ending of their elution were not necessary.

The adsorption and elution characteristics of histidine and arginine were relatively easy to observe by the specific Pauly diazo reaction for histidine and Sakaguchi reaction for arginine. The behavior of lysine and each of the nonbasic amino acids was determined by the nonspecific ninhydrin colorimetric reaction.

Preliminary adsorption trials showed that the basic amino acids, particularly histidine, were not adsorbed completely by the untreated adsorbent mixture. Complete adsorption was possible when the column was pretreated with solutions of hydrochloric, sulfuric, or acetic acid.

Preliminary trials with amino acid mixtures, prepared as stated above, showed that all the amino acids were adsorbed when the mixtures were passed through columns of Lloyd's reagent. However, all the nonbasic amino acids except cystine were separated completely from the basic ones by developing the columns with hydrochloric acid. Cystine was completely separated from the basic amino acids when it was reduced to cysteine before chromatographing, by addition of zinc dust to the amino acid solution acidified with hydrochloric acid. The adsorption and elution characteristics of the nonbasic amino acids were determined by chromatographing each of the nonbasic amino acids with lysine.

Many possible eluants were tested for their effect on the elution characteristics of lysine, histidine, and arginine. The only solvents that were tried in this investigation were water and solvents completely miscible with water. The eluants tested may be classified as follows: strong and weak bases, strong and weak acids, salts, and buffer solutions.

The stronger basic compounds such as sodium hydroxide could not be used with Lloyd's reagent because their passage through the column was too slow.

Sodium bicarbonate proved the most useful of the weaker basic compounds, as it facilitated complete separation of histidine from arginine and removed most of the histidine before elution of lysine began.

Of the acids tested, hydrochloric acid gave the only useful separation. It eluted lysine completely before any histidine was removed and removed much of the histidine before elution of arginine began. However, complete separation of histidine and arginine was not possible.

Salts such as sodium chloride eluted in the same order as did hydrochloric acid, but the elutions were not complete nor the separations clear cut.

The elution powers of pyridine were so general that it was useful only when a single amino acid was on the column or when group elution was desirable.

Of the solvents miscible with water, methanol gave an unexpected elution. Methanol solutions of hydrochloric acid eluted arginine before lysine and histidine, but the separation was not clear-cut.

A study of the elution properties of the various compounds tested suggested a logical separation procedure. Since hydrochloric acid gave the greatest spread among lysine, histidine, and arginine, and removed lysine completely before removing any histidine or arginine, it was used as the first eluant. Sodium bicarbonate was used as the second eluant because it completely separated histidine from arginine. Arginine was removed with pyridine.

PROCEDURE FOR QUANTITATIVE SEPARATION AND DETERMINATION OF BASIC AMINO ACIDS

Make the amino acid solution, 10 ml. of which contains 1 to 6 mg. of lysine, 1 to 4 mg. of histidine, and 1 to 8 mg. of arginine,

about 0.3 N with hydrochloric acid, add 30 to 50 mg. of zinc dust for each 10 ml. of solution, heat to 80° C., and cool.

Insert a 400 by 19 mm. chromatographic tube into the top of a bell jar and attach the bell jar to a vacuum line with a pressure of 30 to 40 mm. of mercury. Push a small wad of cotton to the bottom of the tube, pour 20 grams of absorbent mixture (one-third Lloyd's reagent and two-thirds Hyflo Super-Cel by weight) into the tube, tap the side of the tube to pack the column, and place 0.6 cm. (0.25 inch) of Hyflo Super-Cel on top of the absorbent in the tube.

Pass the following liquids through the column in the order given: 50 ml. of 1.7 N hydrochloric acid, 10 ml. of the amino acid solution, 180 ml. of 0.5 N hydrochloric acid (use first 10 ml. to rinse the amino acid solution onto the absorbent), 200 ml. of 1.0 N hydrochloric acid, 150 ml. of 0.125 M sodium bicarbonate, 100 ml. of 10% pyridine in 0.7 N hydrochloric acid, and 40 ml. of 0.5 N hydrochloric acid. Add a new solution just as the last of the previous solution is about to disappear onto the absorbent.

Cuts were made when 80, 275, 480, 625, and 730 ml. of eluant had passed into the absorbent. The first fraction was discarded. The second fraction contained the nonbasic amino acids and ammonia, the third contained lysine, the fourth contained histidine, and the fifth contained arginine.

Run a blank column, in which 0.3 N hydrochloric acid treated with zinc replaces the amino acid solution, for each set of determinations. This serves as a reagent blank for the individual amino acid determinations.

The absorption and elution procedure requires from 4 to 5 hours.

Since each basic amino acid occurs singly in a separate fraction, it should be possible to determine each one by any reliable method. In this investigation the following procedures, which have been applied routinely in this laboratory, were used.

Neutralize the histidine fraction to litmus with hydrochloric acid and make to volume. Determine the histidine quantitatively by a modification of Pauly's diazo reaction (*9*).

Neutralize the arginine fraction to litmus with sodium hydroxide and make to volume. Determine the arginine quantitatively by a modification of Sakaguchi's reaction (*4*) (use one 25-ml. flask in place of the three 10-ml. flasks called for).

Make the lysine fraction just acid to phenolphthalein with sodium hydroxide. Add 15 ml. of a pH 7.4 buffer solution (50 ml. of 0.4 M potassium dihydrogen phosphate and 4.1 ml. of 1.0 N sodium hydroxide diluted to 100 ml.) and make the solution to volume. Determine lysine quantitatively by the authors' modification of the ninhydrin colorimetric method: Pipet an aliquot containing 0.02 to 0.10 mg. of lysine into a 25-ml. flask and make to 3 ml. with the blank solution. Add 1 ml. of the ninhydrin solution (5 mg. of triketohydrindene hydrate, 0.4 mg. of sodium hydroxide, and 263 mg. of sodium chloride per milliliter) and 5 ml. of glycerol (added with a large-bore pipet), mix the solution thoroughly, and place in a boiling water bath. After 30 minutes remove the solution, cool, and make to volume with 95% ethanol. Within 15 minutes determine the transmission value of the colored solution in a photoelectric colorimeter and evaluate the amount of lysine by reading this value from a standard curve prepared by using known amounts of lysine in the procedure outlined above.

The transmission values of the colored solutions can be determined in any photoelectric colorimeter with which a spectral region with a maximum transmission at 5400 Å. can be isolated. In this investigation a KWSZ photoelectric photometer was used with Corning filters 978 and 351, 1-cm. adsorption cells, 5% copper sulfate in the cooling cells, and 67.5 volts on the photocell circuit.

The lysine in the lysine fractions from some of the protein hydrolyzates was also determined by the total amino nitrogen method (*13*), by the α-amino nitrogen method (*14*), and by the method involving the difference between the total and α-amino nitrogen (*14*).

The proteins used in this investigation were hydrolyzed with 20 ml. of 20% hydrochloric acid for 18 hours. The hydrolyzates were filtered, concentrated under vacuum, and reconcentrated three times after addition of water to remove the hydrochloric acid. One milliliter of concentrated hydrochloric acid was added and the solutions were made to 50 ml. When decolorizations were made, acid- and alkali-washed Norit was used. The nucleic acid used in the experimental work was treated in the same manner as the proteins.

RESULTS AND DISCUSSION

Recoveries of lysine, histidine, and arginine adsorbed from amino acid mixtures were excellent (Table I). The slightly high recovery of lysine may have been due either to lysine impurities in the other amino acid preparations or to traces of nonbasic amino acids that escaped removal before elution of lysine began.

The amounts of the basic amino acids found in several proteins compare fairly well in most cases with those which Block (*3*) considers "best values" (Table II), especially since different preparations of the same protein may vary somewhat in their analysis. Since the lysine values were higher than most reported values, it is possible that other ninhydrin-color-producing substances were present in the lysine fraction. To check this possibility the amounts of lysine present in the lysine fractions of hydrolyzates from several proteins were determined by the ninhydrin colorimetric method and by amino nitrogen determinations (Table III). Since the errors in the amino nitrogen determinations may be high when small amounts of lysine are determined, the comparisons are considered good. The results indicate that only traces of interfering substances could have been present in the lysine fraction.

Stanley (*10*) reported no lysine present in tobacco mosaic virus. Knight (*8*) found 1.35% lysine in the virus, but decided his findings were inconclusive, since he could not isolate any lysine by

Table I. Recovery of Lysine, Histidine, and Arginine from a Mixture of Amino Acids[a] and Ammonium Chloride

Amino Acid	Amount in Solution	Amount in Eluate	Recovery
	Mg.	*Mg.*	%
Lysine	4.00	4.04	101.0
	4.00	4.21	105.3
	4.00	4.13	103.3
	1.00	1.01	100.3
Histidine	4.00	3.97	99.3
	4.00	4.01	100.3
	4.00	4.04	101.0
	1.00	0.99	99.0
Arginine	8.00	7.76	97.0
	8.00	8.08	101.0
	8.00	7.90	98.8
	2.00	1.96	98.0

[a] Tyrosine, tryptophane, methionine, glutamic acid, aspartic acid, cystine, glycine, alanine, valine, norleucine, isoleucine, leucine, phenylalanine, threonine, histidine, arginine, lysine.

Table II. Basic Amino Acid Content of Proteins[a]

(16% nitrogen basis)[b]

Proteins	Nitrogen	Lysine		Histidine		Arginine	
		Adsorption value[c]	"Best value"[d]	Adsorption value[c]	"Best value"[d]	Adsorption value[c]	"Best value"[d]
	%	%	%	%	%	%	%
Casein (Pfanstiehl)	13.6	8.6	7.5	3.3	2.5	3.7	4.2
Fibrin (C.P., City Chemical Corp.)	13.2	9.1	7.5	2.7	2.4	6.9	7.8
Lactalbumin (Labco, Borden)	13.6	11.2	9.6	2.2	2.0	3.4	3.9
Egg albumin (C.P., City Chemical Corp.)	12.3	8.0	5.0	2.7	2.0	6.0	5.8
Blood albumin (C.P., City Chemical Corp.)	11.3	9.7	10.0	3.2	2.8	5.7	5.5
Gelatin (Sargent)	16.1	4.5	4.5	0.75	0.8	8.0	8.0
Tobacco mosaic virus (Stanley)	?	1.42	0.0[e]	0.0	0.0[e]	9.3	9.0[e]

[a] Protein hydrolyzates not treated with Norit.
[b] Values calculated to 16% nitrogen basis for comparison only.
[c] Values did not vary more than ±3%.
[d] Block and Bolling (*3*).
[e] Stanley (*10*).

Table III. Comparison of Methods for Determining Lysine in Lysine Fraction from Protein Hydrolyzates (Norit-Treated)

Protein	Lysine by Ninhydrin Colorimetric Method	Lysine by Total Amino Nitrogen Method	Lysine by α-Amino Nitrogen Method	Lysine by Total Amino Nitrogen Minus α-Amino Nitrogen Method
	%	%	%	%
Casein	7.3	7.2	7.5	7.0
Fibrin	7.5	7.8	7.6	8.0
Lactalbumin	9.3	9.5	9.4	9.7
Egg albumin	5.9	6.1	6.1	6.2
Blood albumin	6.5	6.5	6.5	6.6
Gelatin	4.5	4.6	4.9	4.4
Corn 1	0.23	0.23	0.23	0.23
Corn 2	0.26	0.26	0.29	0.22
Corn 3	0.25	0.28	0.26	0.29
Corn 4	0.24	0.23	0.27	0.19
Corn average	0.25	0.25	0.26	0.23

precipitation methods. Stokes *et al.* (*11*) by use of microbiological techniques found 1.36% lysine in the virus. The 1.42% found by the adsorption method is in good agreement with Knight's and Stokes's findings. All the above determinations were made on tobacco mosaic virus prepared by Stanley.

The histidine values might also be questionable, since there are other naturally occurring basic compounds that give a color with Pauly's diazo reaction. The compounds most likely to be present in quantities sufficient to cause interferences are the purine and pyrimidine bases occurring in nucleic acids. The values for histidine, arginine, and lysine were not affected when hydrolyzed yeast nucleic acid, in amounts several times higher than would occur in proteins, was added to the amino acid solutions before chromatographing (Table IV).

Decolorization of the acid hydrolyzates affected slightly the basic amino acid values, particularly histidine and arginine values (Table V). However, it was found that this preparation of Norit adsorbed slight amounts of the basic amino acids. The variation of values from the treated samples were greater in some cases than the values from the untreated samples. Even though there was some color removed during the elution of histidine, this did not account for the differences obtained in the histidine values. It is recommended that the protein hydrolyzates be chromatographed without decolorization.

The values for arginine and histidine found in some corn grain hydrolyzates are compared to values obtained from the same samples by Doty *et al.* (*5*) by use of phosphotungstic acid to separate the basic amino acids before determination of histidine and arginine (Table VI). There were no corrections made for the solubility of the phosphotungstates, which accounts, at least in part, for the lower values obtained by the phosphotungstic acid precipitation method.

Table IV. Effect of Nucleic Acid on Basic Amino Acid Values[a]

Protein	Lysine		Histidine		Arginine	
	No nucleic acid	100 mg. of nucleic acid	No nucleic acid	100 mg. of nucleic acid	No nucleic acid	100 mg. of nucleic acid
	%	%	%	%	%	%
Lactalbumin	9.4	9.3	1.8	1.8	2.8	2.8
Egg albumin	6.2	6.3	2.1	2.1	4.6	4.7
Blood albumin	7.1	7.1	2.2	2.2	3.8	3.8

[a] Protein hydrolyzates treated with Norit.

Table V. Effect of Decolorization of Protein Hydrolyzates with Norit on Basic Amino Acid Values

Protein	Lysine		Histidine		Arginine	
	Untreated	Treated	Untreated	Treated	Untreated	Treated
	%	%	%	%	%	%
Casein	7.4	7.6	2.8	2.8	3.2	3.2
Fibrin	7.7	7.7	2.3	2.2	5.6	5.6
Lactalbumin	9.7	9.4	1.9	1.8	2.9	2.8
Egg albumin	6.3	6.2	2.2	2.1	4.9	4.6
Blood albumin	7.0	7.1	2.3	2.2	4.1	3.8
Gelatin	4.6	4.5	0.78	0.64	8.1	7.5

CONCLUSIONS AND SUMMARY

The individual basic amino acids have been separated chromatographically from amino acid mixtures and protein hydrolyzates by use of Lloyd's reagent. This is the first time that a single adsorption has been used successfully for the separation of the basic amino acids. The recoveries of the basic amino acids when they were adsorbed from amino acid mixtures were 103 ± 2% for lysine, 100 ± 1% for histidine, and 99 ± 2% for arginine. The method is simple, rapid, and reliable. Arginine was determined by a modification of the Sakaguchi reaction and histidine by a modification of Pauly's diazo reaction. Lysine was determined by a modification of the ninhydrin colorimetric reaction which was standardized for quantitative determination. Evidence indicates that substances from protein hydrolyzates that may interfere with this reaction were absent. Accurate determination of lysine in protein hydrolyzates was possible even when the percentage of this amino acid was small. Nucleic acid did not interfere with the adsorption or subsequent determination of the basic amino acids.

Table VI. Basic Amino Acids in Corn

Sample	Nitrogen	Histidine		Arginine		Lysine
		Adsorption value	PTA value	Adsorption value	PTA value	Adsorption Value
	%	*Mg./g.*		*Mg./g.*		*Mg./g.*
1	1.50	2.9	2.0	3.9	3.6	2.3
2	1.59	2.7	2.2	4.0	4.1	2.6
3	1.45	2.5	2.0	3.7	3.8	2.5
4	1.45	2.6	2.3	4.0	3.9	2.4

Values of the basic amino acids determined in seven protein hydrolyzates were compared to literature values. The lysine and histidine values obtained by the adsorption method were generally higher than the average of the reported values, but the adsorption values checked more closely with the most recent literature values. The adsorption values for arginine compared satisfactorily with the average literature values.

The basic amino acids were determined in four corn grain hydrolyzates. The values for arginine and histidine were compared to values obtained when the basic amino acids were separated from hydrolyzates from the same corn grain samples by phosphotungstic acid precipitation. The arginine values checked very closely, but the adsorption values for histidine were appreciably higher than those obtained after phosphotungstic acid precipitation.

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Aids to Computation in Spectrophotometric Analysis of Binary Mixtures

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Several methods are described for facilitating the computation of concentrations of binary mixtures from the observed spectrophotometer data. The methods are especially useful for cases of Beer's law failure. One is a graphical method in which the observed optical densities of a mixture determine the location of a point inside a coordinate network reading directly in concentrations. The other method utilizes a specially constructed slide rule on which concentrations are read when the position is adjusted according to the densities. Illustrative data for benzene-toluene mixtures are shown. The saving in time over numerical calculation can amount to more than a factor of 20. The graphical method can be extended to three-component mixtures.

THE spectrophotometric analysis of mixtures of absorbers requires measurement of the optical density at selected wavelengths and solution of the resulting simultaneous equations. These equations are nonlinear in cases in which there is an apparent failure of Beer's law and are consequently tedious to solve directly. A method of solution based on successive approximations has been described (*1*). The present paper describes several devices which have been used for some time to shorten the labor of computation for binary mixtures.

An example which has been of considerable practical importance in the production of nitration grade toluene is the analysis of benzene-toluene mixtures in a transparent solvent such as isooctane.

For this case, using a particular set of experimental conditions (constants must be determined for every optical system used) and assuming no Beer's law failure, the following equations apply:

$$D^{268.9} = 1.01\ C_b + 20.8\ C_t \quad (1)$$

$$D^{260.9} = 16.3\ C_b + 19.4\ C_t \quad (2)$$

where the optical densities, D, are measured at characteristic absorption peaks at the indicated wave lengths expressed in millimicrons (the peaks for the two compounds happen almost to coincide in wave lengths but not in intensities and for the author's work were found most sensitive to relative composition) and the concentrations, C, are given in volume per cent. Equations 1 and 2 may be transformed to the more convenient form:

$$C_b = 0.0651 D^{260.9} - 0.0607\ D^{268.9} \quad (3)$$

$$C_t = -0.0032\ D^{260.9} + 0.0510\ D^{268.9} \quad (4)$$

GRAPHICAL SOLUTION

These equations may be thought of as defining a transformation from D axes to C axes, as illustrated in Figure 1. Here the densities at 268.9 mμ for various concentrations of the pure compounds in a transparent solvent are plotted as a function of the densities at 260.9 mμ, the toluene axis having approximately unit slope because the peaks are about of equal intensity, while the slope for the benzene axis is much lower because the 268.9 peak is much weaker than that at 260.9 for this compound. The densities of a mixture are represented by a point somewhere inside these two lines. It will be seen that, speaking in the language of vectors, the components along the concentration axes of a point defined by components along the density axes can be obtained graphically by projecting back parallel to the concentration axes.

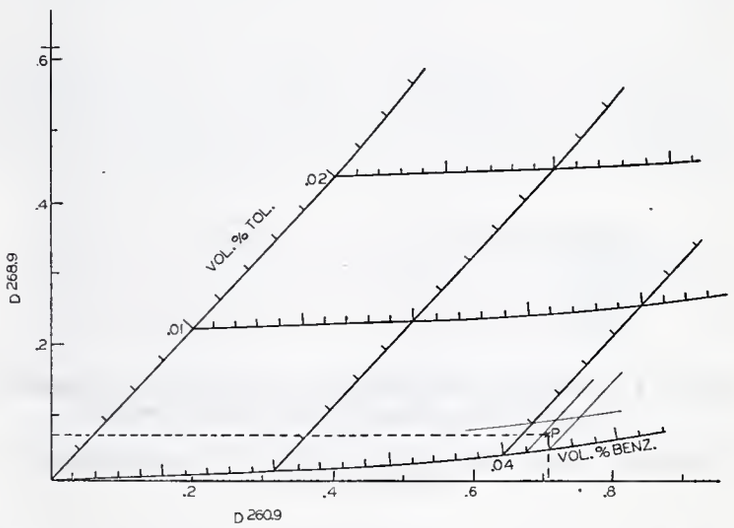

Figure 1. Graphical Method of Obtaining Concentrations of Benzene-Toluene Mixtures from Observed Optical Densities

Example shown at *P* represents mixture 1 of Table I

The network consisting of the concentration axes repeated at regular intervals of benzene and toluene concentration, as indicated in Figure 1, is drawn in merely for convenience. Concentrations can be obtained in this way in a much shorter time than by numerical solution of Equations 3 and 4. The chief advantage, however, is found in the case in which the densities are not linear functions of the concentrations. This situation arises when benzene-toluene mixtures are measured on a Beckman quartz spectrophotometer, which requires an effective slit width (0.30 mm. corresponding to about 0.8 mμ) comparable with the width of the absorption bands. In this case the data can be represented by a curvilinear coordinate system for the concentration axes which is a distortion of the previous case, the concentrations being obtained by following a curvilinear network. Figure 1 actually rep-

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resents Beckman spectrophotometer data. It will be seen that the curvature of the axes is not great but cannot be ignored for accurate work. Linear interpolation between any two successive 0.01% intervals on the concentration scales is satisfactory.

SLIDE RULE SOLUTION

Another device which has been found convenient in practice is a slide rule, Figure 2, with appropriate scales reading directly in concentrations. These scales are drawn on strips of paper which are pasted on a conventional slide rule and covered with label varnish. The action of the rule depends on the fact that each concentration in Equations 3 and 4 is expressed as the difference between two linear terms, so that if the density scales on the rule are each drawn with units adjusted in length according to the corresponding coefficients the subtractions can be performed mechanically. The operation is thus analogous to the operation of division on a conventional slide rule and requires the same length of time to obtain each concentration as a simple division. The rule has one density scale for 268.9 mμ but a separate density scale for 260.9 mμ is used for each compound. Based on the data of Equations 3 and 4 and assuming no Beer's law failure, unit length on the $D^{260.9}$ scale used for the toluene determination would be 0.0032/0.0510 the unit length on the $D^{268.9}$ scale, while unit length on the $D^{260.9}$ scale used for the benzene determination would be 0.0651/0.0607 that of the $D^{268.9}$ scale. The concentration scales are not in equivalent positions on the rule because the algebraic signs of the coefficients in Equations 3 and 4 are interchanged. In operation the movable hair line is set at the observed value on one of the $D^{260.9}$ scales, the $D^{268.9}$ scale moved to read the observed value for this wave length at the hair line, and the concentration in volume per cent read opposite the index arrow.

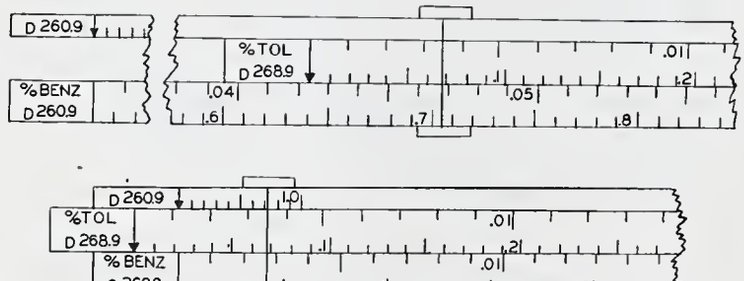

Figure 2. Slide Rule for Obtaining Concentrations of Benzene-Toluene Mixtures from Observed Optical Densities

Setting shown illustrates (above) benzene and (below) toluene determination for mixture 1 of Table I

The construction of the slide rule for the nonlinear case which is actually represented in Figure 2 is not quite so simple and is best carried out with the aid of the corresponding graph—i.e., Figure 1. The following procedure has been found to yield exact results, although other choices could doubtless be made. (It is possible that this method of attack might not be valid in all cases.)

The $D^{260.9}$ scale used for the determination of benzene is laid out linearly as before. With the zero of the movable scale in coincidence with that of the fixed scale, the $D^{268.9}$ scale is laid out from the readings of the toluene axis of the graph—i.e., suitable increments of the $D^{268.9}$ scale are drawn in opposite the associated values of the $D^{260.9}$ scale. For example, 0.2 on the $D^{268.9}$ scale would be placed opposite point P corresponding to the associated density on the $D^{260.9}$ scale. The movable hair line and the movable scale are then adjusted to correspond to associated densities for various concentrations of pure benzene, as obtained from the benzene axis, and the values for the C_b (% benzene) scale drawn in opposite the index arrow for each setting. The same procedure is now followed for the toluene scales, using the $D^{268.9}$ scale already obtained, drawing in another $D^{260.9}$ scale from data from the benzene axis with the zero points in coincidence, and then laying out the C_t (% toluene) scale from associated densities obtained from the toluene axis.

Table I. Comparison of Different Procedures for Converting Observed Densities of Benzene-Toluene Mixtures to Concentrations

Mixture No.	Constituent	Volume Per Cent Known	Shell	Graph	Rule
1	Benzene	0.0424	0.0421	0.0423	0.0423
	Toluene	0.0012	0.0014	0.0012	0.0012
	Total	0.0436	0.0435	0.0435	0.0435
2	Benzene	0.0290	0.0288	0.0288	0.0289
	Toluene	0.0060	0.0060	0.0059	0.0060
	Total	0.0350	0.0348	0.0347	0.0349
3	Benzene	0.0122	0.0121	0.0121	0.0121
	Toluene	0.0121	0.0122	0.0122	0.0122
	Total	0.0243	0.0243	0.0243	0.0243
4	Benzene	0.0116	0.0114	0.0114	0.0113
	Toluene	0.0194	0.0195	0.0196	0.0194
	Total	0.0310	0.0309	0.0310	0.0307
5	Benzene	0.0081	0.0078	0.0076	0.0075
	Toluene	0.0421	0.0424	0.0427	0.0424
	Total	0.0502	0.0502	0.0503	0.0499
6	Benzene	0.0033	0.0033	0.0033	0.0032
	Toluene	0.0168	0.0168	0.0168	0.0168
	Total	0.0201	0.0201	0.0201	0.0200
	Benzene	0.0018	0.0019	0.0020	0.0018
	Toluene	0.0388	0.0386	0.0386	0.0387
	Total	0.0406	0.0405	0.0406	0.0405
	Benzene	0.0007	0.0007	0.0007	0.0006
	Toluene	0.0155	0.0154	0.0155	0.0155
	Total	0.0162	0.0161	0.0162	0.0161

The resulting slide rule is similar to that used in the case of no Beer's law failure, except that only one scale is linear, the others converging or diverging. Linear interpolation on these scales between any two successive 0.01% concentration or 0.1 density points is convenient and satisfactory.

COMPARISON OF METHODS

A comparison of the different methods of treating the same data is shown in Table I. The Shell method refers to the successive approximation procedure described in (*1*). The results reported under graph were obtained with a curvilinear graph 50 × 50 cm., while a 50-cm. (20-inch) slide rule was used for those listed under rule. The average time required for obtaining both the benzene and toluene concentrations from the observed densities for a given mixture is approximately 5 to 8, 0.5, and 0.3 minute for the respective methods. The construction of the graph requires 3 or 4 hours, and the slide rule scales require about 2 working days.

THREE-COMPONENT MIXTURES

Linear equations for three-component mixtures may be solved with a two-dimensional graph by using as one of the equations the condition that the sum of the concentrations is known. The optical densities at any one wave length may be indicated on a conventional triangular composition diagram as a contour map. It will be found that lines of constant density for different compositions are straight parallel lines. For another wave length the constant density lines will have a different slope and different spacing for the same density interval. The composition is determined by the point of intersection of the measured density lines at the two wave lengths. The method is more rapid than numerical solution. It has not been tested for cases of Beer's law failure but should also apply if the deviations are not so large as to introduce contour curvatures with more than one point of intersection.

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Conditions Affecting the Sequence of Organic Compounds in Tswett Adsorption Columns

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TSWETT'S columnar or chromatographic adsorption method is particularly useful for the resolution of mixtures of organic compounds and for the detection and estimation of the individual components. Under given conditions, compounds adsorbed in the columns form a series of bands or zones in definite sequence. As a result, organic substances are frequently identified and named with respect to their relative positions in the adsorption columns (*13*, *15*). In several instances, prognostications regarding chemical structures of organic compounds have been based upon their adsorbability relative to that of substances of known constitution (*9*).

This sequence or adsorption order of the bands is, however, not the same under all circumstances; it varies with the conditions in the columns, with the solvent and the adsorbent, and with the kinds of substances adsorbed (*9*, *10*). Variations of the adsorption sequence have been observed most frequently when mixtures of dissimilar compounds are adsorbed under different conditions (*1*, *6*, *9*). For example, mixtures of the green chlorophylls and the yellow xanthophylls isolated from the green parts of plants often yield different adsorption sequences (*10*). Variations of the adsorption sequence have also been observed when mixtures of similar substances, such as the xanthophylls themselves, are resolved by adsorption (*13*).

Further investigation of the relative adsorbability of organic compounds has now revealed many additional examples of variation of the adsorption sequence. These variations have resulted from alteration of one or more factors such as the solvent, the adsorbent, the temperature, the concentration, the presence of an impurity, or the concentration of hydrogen ion. As experience is gained, additional conditions which influence the adsorption sequence will undoubtedly be found.

The number of possible adsorption sequences is related to the number of components in the mixtures that are adsorbed. With a binary mixture, two sequences of the bands are possible. Under certain conditions, one solute will form the upper band; under other conditions, this solute may form the lower band. As in a series of permutations, increase in the number of components of a mixture multiplies the number of possible sequences of the adsorption bands. For 3 components there are 6 possible adsorption sequences; with 4 components there are 24 possible sequences; with 5 components there are 120 sequences; and for 10 components there are 3,628,800 possible sequences. Only with binary mixtures have all the theoretically possible sequences been obtained.

Alteration of the adsorption sequence may be utilized to increase the sensitivity and to broaden the applicability of the chromatographic adsorption method. Variation of the adsorption sequence with conditions in the columns illustrates precautions to be observed in new applications of the columnar adsorption technique.

MATERIALS AND METHODS

Effects of various conditions upon the adsorption sequence were determined by use of colored solutes, which are readily visible in adsorption columns. These mixtures were made of chlorophylls, of chlorophylls and xanthophylls, of xanthophylls, or of common water-soluble dyes.

Pigments and Their Sources. Because of their lability, chlorophylls and xanthophylls were prepared in small quantities as needed. Chlorophylls *a* and *b* were isolated by adsorption of the freshly prepared extracts of green leaves (*11*). The labile isomer of each of these pigments, chlorophyll *a'* and chlorophyll *b'*, was obtained by adsorption of extracts of heated leaves (*12*). Chlorophyll *d* was prepared from red algae (*Rhodophyceae*) (*7*).

Xanthophylls, the alcohol-soluble oxygen derivatives of polyene hydrocarbons, were extracted from a variety of plant sources and were isolated by adsorption. Lutein and neoxanthin were obtained from leaves, fucoxanthin from the kelps or brown seaweeds (*Pheophyceae*), diadinoxanthin and peridinin (sulcatoxanthin) from the symbiotic alga of a sea anemone, diatoxanthin from diatoms, violaxanthin from pansies, and taraxanthin and tareoxanthin from dandelions (*13*). Zeaxanthin was isolated from the fruit pods of the Chinese lantern (*Physalis*) (*9*). On the Pacific Coast, sources of many of these pigments are available throughout the year.

Solvents and Adsorbents. Solvents were purified by distillation in an all-glass apparatus. Commercial adsorbents were employed, usually without further treatment.

Heat-treated siliceous earths, Hyflo Super-Cel and Celite 501, 503, 535, and 545, from Johns-Manville, were all weakly adsorptive (comparable in adsorptive capacity to powdered sugar). All these products are manufactured by the same alkali flux calcination process and differ principally in particle size.

Powdered sugar (confectioners' powdered sugar, grade XXXXXX, containing 3% starch to prevent caking), which was obtained from several sources, exhibited rather uniform adsorptive properties. Cellulose was prepared for use in columns by disintegration of finely cut filter paper in water in a Waring Blendor. Preparations of activated magnesium silicate of similar adsorptive properties but of greatly different particle size were obtained from three sources: Florisil from the Floridin Company, Warren, Pa.; Magnesol from Westvaco Chlorine Products Corporation, Newark, Calif., and magnesium silicate No. 34 from Philadelphia Quartz Company Limited, Berkeley, Calif. Activated magnesium oxide, Micron Brand No. 2641 from Westvaco Chlorine Products Corporation, was mixed with Celite before use in columns (*8*).

Procedure. Solutions of the mixtures of plant pigments were prepared by dissolving 1 to 5 mg. of each substance in 100 ml. of petroleum ether. These solutions were then filtered through adsorption columns (1.5 cm. in diameter and 12 to 20 cm. long) prepared by pressing the dry adsorbent into the tube. As soon as a narrow zone (0.3 to 1 cm.) at the top of the adsorbent was saturated with pigment, the adsorbed materials were washed with fresh solvent (or solvent mixture) in order to develop the chromatogram and to reveal the adsorption sequence.

Water-soluble dyes were dissolved directly in water or in acidic or alkaline solution. Concentration of the dyes in these solutions was comparable to that of the plant pigments in the petroleum ether solutions.

CONDITIONS INFLUENCING ADSORPTION SEQUENCE OF COMPONENTS OF BINARY MIXTURES

Adsorption from Different Solvents. In columns of a given adsorbent, adsorption sequences are determined, in part, by the properties of the solvent. There are many examples of a chlorophyll and a xanthophyll adsorbed in two sequences when different solvents are used.

With petroleum ether or benzene as solvent and with Celite as adsorbent, chlorophyll *b* forms a yellow-green band above the yellow band of neoxanthin. Addition of about 25% of acetone to the petroleum ether or benzene causes the chlorophyll *b* to be adsorbed below the neoxanthin.

With petroleum ether, benzene, or 1,2-dichloroethane as solvent, chlorophyll *d* is adsorbed above fucoxanthin in columns of powdered sugar. If 0.5 to about 3% of an alcohol is added to these solvents, chlorophyll *d* is adsorbed below fucoxanthin.

When petroleum ether containing about 5% acetone is used as solvent, chlorophyll *a* is adsorbed above lutein in columns of

Celite. The presence of about 0.75% ethanol in the petroleum ether plus acetone causes the chlorophyll *a* to be adsorbed below the lutein.

Many similar alterations of the adsorption sequence have been produced by adsorption of binary mixtures of chlorophyll *a* and a xanthophyll. When petroleum ether is the solvent and powdered sugar is the adsorbent, chlorophyll *a* is adsorbed above each of the following xanthophylls: violaxanthin, fucoxanthin, diadinoxanthin, tareoxanthin, taraxanthin, diatoxanthin, zeaxanthin, and lutein. With petroleum ether containing 0.5 to 1% propanol as solvent, chlorophyll *a* is adsorbed below each of these xanthophylls.

Substances of the same type, such as the xanthophylls themselves, are often adsorbed in different sequences when different solvents are used with columns of a given adsorbent. With chloroform as solvent, zeaxanthin is adsorbed below violaxanthin in columns of magnesia plus Celite (1 to 1). With petroleum ether plus about 25% of ketones (such as acetone, ethyl methyl ketone, or methyl nonyl ketone) as solvent, zeaxanthin is adsorbed above violaxanthin.

With 1,2-dichloroethane as solvent, fucoxanthin is adsorbed above violaxanthin in columns of sugar. But with petroleum ether plus about 0.5% of an alcohol such as methanol, ethanol, propanol, or butanol as the solvent, fucoxanthin is adsorbed below violaxanthin.

Variations of the proportions of a mixture of solvents may also suffice to alter the sequence of substances adsorbed on a given adsorbent. When petroleum ether plus about 5% acetone and 0.5% ethanol is used as solvent, fucoxanthin is adsorbed above chlorophyll *a* in columns of Celite. Increase of the alcohol concentration to about 2% causes the fucoxanthin to be adsorbed below the chlorophyll *a*. At one intermediate concentration of alcohol, fucoxanthin and chlorophyll *a* are difficultly separable in the columns of Celite.

Impurities in Solvents. Very small quantities of alcohols which may occur as impurities in a solvent often alter the sequence of adsorbed chlorophylls and xanthophylls. In columns of sugar, where fucoxanthin is normally adsorbed below chlorophyll *a* when petroleum ether is used as solvent, as little as 0.05% amyl alcohol in the petroleum ether causes the fucoxanthin to be adsorbed above the chlorophyll *a*. A similar effect is produced by as little as 0.1% cholesterol or ergosterol dissolved in the petroleum ether. Unsaturated hydrocarbons, chlorinated hydrocarbons, ethers, esters, organic acids, and amides of organic acids added to petroleum ether accelerate the movement of fucoxanthin and chlorophyll *a* through columns of sugar, but the adsorption order is identical with that produced by petroleum ether alone.

Addition of very small quantities of alcoholic substances to a solution of fucoxanthin and chlorophyll *a* in petroleum ether before adsorption in a column of sugar causes the chlorophyll to form the lower band. But as the column is washed with fresh, unadulterated portions of petroleum ether, the fucoxanthin gradually becomes adsorbed below the chlorophyll *a*.

Alcohols are common in plants, and it seems probable that they may cause the reversal of the adsorption order of chlorophylls and xanthophylls which occurs when extracts of plants are adsorbed. When a petroleum ether extract of the brown seaweeds is filtered through a column of powdered sugar, chlorophyll *a* is adsorbed below fucoxanthin, the principal xanthophyll. As the chromatogram is developed with fresh petroleum ether, fucoxanthin slowly appears below the chlorophyll *a*. In analogous fashion, a petroleum ether extract of the leaves of higher plants yields chlorophyll *a* adsorbed below the principal xanthophyll lutein. Development of the chromatogram with petroleum ether carries the lutein below the chlorophyll *a*.

Use of Different Solvents for Development of One Chromatogram. As indicated by the adsorption of pigments from solutions containing impurities, bands of two substances separated in one sequence by adsorption from one solvent ma have their relative positions reversed by further development c the chromatogram with another solvent. Because the resolutio effected by the first solvent must be overcome by the secon solvent in the lower unused portions of the adsorbent, long ac sorption columns are usually required.

If a mixture of chlorophyll *a* and zeaxanthin in solution in d chloroethane is adsorbed on a column of sugar, zeaxanthin forn the lower band. Further development of the chromatogra with petroleum ether containing about 0.5% ethanol causes th chlorophyll *a* to overtake and to pass through the zeaxanthi band, thus reversing the adsorption sequence. Through th alternate use of these two different solvents in a long column c sugar, the adsorption sequence of chlorophyll *a* and zeaxanthi may be reversed several times.

Mixtures of either chlorophyll *a*, *b*, or *d* with one xanthophy have also been separated in first one sequence and then in th other by the successive use of different solvents for developmen of the chromatogram. Mixtures of two xanthophylls have lik wise been separated in one sequence and then in the other by de velopment of the chromatogram with two different solvents. Thi may be illustrated by the adsorption of taraxanthin and lutei in columns of a mixture of magnesia and Celite. With dichloro ethane as solvent, lutein forms a band below that of taraxanthin When the column is subsequently washed with petroleum ethe containing about 25% acetone, taraxanthin advances throug the band of lutein. Conversely, lutein first separated belo taraxanthin by adsorption on magnesia from solution in pe troleum ether plus acetone can be made to form the upper ban by washing the column with 1,2-dichloroethane.

Effect of Hydrogen Ion. Different concentrations of hy drogen ion in aqueous solution may cause mixtures of water soluble substances to separate in different sequences. As a example, on columns of cellulose bromothymol blue (dibromo thymol sulfonephthalein) is adsorbed below fluorescein when th solutions are alkaline (ammoniacal). From neutral, buffere solutions or acidic solutions, however, bromothymol blue is ad sorbed above fluorescein.

Concentration of Solutes. At higher concentrations, a sol ute usually migrates through an adsorption column faster tha at lower concentrations. For this reason, increase in the concen tration of a more adsorbed constituent of a binary mixture ma cause the band of this more adsorbed component to advance be yond the band of the less adsorbed component. From dilut ammoniacal solution, fluorescein is slightly more adsorbed tha bromothymol blue in columns of cellulose (and in strips of filte paper) when about equal quantities of the pigment are adsorbed If the ratio of fluorescein to bromothymol blue is increased t about 50 to 1, fluorescein appears ahead of the bromothymol blue. But the advancing band of fluorescein has a dilute, diffuse, trailing boundary that moves slower than the band of bromothymol blue.

Table I. Adsorption Sequences of Chlorophyll *b*, Chlorophyll *a*, and Fucoxanthin

	Columns of Magnesia and of Celite		
Adsorbents	Magnesia + Celite (1 to 1)	Celite	Celite
Solvents	Petroleum ether + 25% acetone	Petroleum ether + 0.75% ethanol	Petroleum ether + 0.75% ethanol + 5% acetone
Adsorption sequences	Chlorophyll *b* Chlorophyll *a* Fucoxanthin	Fucoxanthin Chlorophyll *b* Chlorophyll *a*	Chlorophyll *b* Fucoxanthin Chlorophyll *a*
	Columns of Sugar		
Adsorbents	Sugar	Sugar	Sugar
Solvents	Petroleum ether + 5% acetone	Petroleum ether + 0.75% ethanol	Petroleum ether + 5% acetone + 0.25% ethanol
Adsorption sequences	Chlorophyll *b* Chlorophyll *a* Fucoxanthin	Fucoxanthin Chlorophyll *b* Chlorophyll *a*	Chlorophyll *b* Fucoxanthin Chlorophyll *a*

As a result, the band of bromothymol blue appears as a blue zone in the middle of a rapidly widening yellow band of fluorescein. If the concentration of the fluorescein is not too great, the original band is not too wide, and the column is long enough, the band of fluorescein widens; its rate of movement decreases; and it gradually falls behind the band of bromothymol blue.

Different Adsorbents. The sequence of two substances adsorbed from a given solvent varies from adsorbent to adsorbent. In columns of Celite, chlorophyll *a* is adsorbed below lutein when petroleum ether plus 0.5% ethanol plus 5% acetone is used as solvent; but in columns of sugar, chlorophyll *a* is adsorbed above lutein. In columns of magnesia, with petroleum ether plus 5% acetone as solvent, chlorophyll *a* is adsorbed above lutein, but in columns of sugar these pigments are adsorbed in the inverse order.

Relative adsorbability of the xanthophylls also varies with the adsorbent. When magnesia plus Celite is the adsorbent and dichloroethane is the solvent, zeaxanthin is adsorbed above fucoxanthin; but in columns of sugar, zeaxanthin is adsorbed below fucoxanthin. From solution in petroleum ether plus about 10% acetone, lutein is adsorbed below taraxanthin in columns of Celite or of powdered sugar; but in columns of magnesia or of magnesia plus Celite lutein is adsorbed above taraxanthin. In this latter example, the resolving power of the magnesia tending to separate the pigments in one sequence is greater than the resolving power of the Celite tending to separate the pigments in the other sequence.

In columns of different adsorbents, water-soluble substances also separate in different sequences. From ammoniacal solution bromothymol blue is adsorbed above fluorescein in columns of Celite or of magnesium silicate. In columns of magnesia plus Celite, in columns of cellulose, or in strips of filter paper (by capillary analysis) bromothymol blue is less adsorbed than fluorescein.

Alteration of the Adsorbent. Treatment of an adsorbent in different ways may serve to invert the sequence of adsorbed substances. In columns of Celite, fucoxanthin is adsorbed above chlorophyll *a* when petroleum ether plus about 0.75% ethanol is employed as solvent. If, however, the Celite is moistened uniformly with water (about 5 to 10% by weight) before it is packed into the adsorption tube, the fucoxanthin is weakly adsorbed, forming a band just below the chlorophyll *a*. By contrast, chlorophyll *a* is adsorbed above fucoxanthin in columns of dry or of moist Celite when petroleum ether plus 5% acetone is used as solvent.

Impurities on an adsorbent sometimes cause substances to be adsorbed in different sequences. Sugar used for adsorption of extracts of plant material and recovered by drying often retains impurities in sufficient quantities to cause chlorophyll *a* to be adsorbed below xanthophylls such as fucoxanthin, zeaxanthin, and lutein (with petroleum ether as solvent), whereas in columns of fresh sugar chlorophyll *a* is adsorbed above these xanthophylls. A similar effect is obtained when sugar that has been washed with alcohol is completely dried before re-use in columns.

Layers of Adsorbents. Layers of different adsorbents in a single adsorption column may cause the bands of adsorbed compounds to alter their relative positions as they are carried through the adsorbent. In a column composed of a deep lower layer of magnesia and a shallower upper layer of sugar, chlorophyll *a* is adsorbed below zeaxanthin in the sugar layer but gradually becomes adsorbed above zeaxanthin in the magnesia layer when petroleum ether plus 0.5% ethanol are employed as the solvent.

Adsorption at Different Temperatures. Variation of the temperature may sometimes change the adsorption sequence. At 95° C., lutein dissolved in decalin plus 0.5% propanol is adsorbed below chlorophyll *a* in columns of powdered sugar. Under similar conditions but at 20° C., lutein is adsorbed above chlorophyll *a*. At the higher temperature, spontaneous isomerization of chlorophyll *a* causes formation of a wide green band which does not separate into distinct bands of chlorophyll *a* and chlorophyll *a'* (*12*).

At 95° C. bromothymol blue and fluorescein, in dilute ammoniacal solution, pass through adsorption columns of magnesium silicate slightly faster than at 20° C. At each temperature, fluorescein forms the lower band.

Table II. Adsorption Sequences

Chlorophyll *b*, Fucoxanthin, and Zeaxanthin

Adsorbents	Sugar	Sugar	Magnesia + Celite (1 to 1)
Solvents	1,2-Dichloroethane	Petroleum ether + 0.5% ethanol	Petroleum ether + 25% acetone
Adsorption sequences	Chlorophyll *b* Fucoxanthin Zeaxanthin	Fucoxanthin Chlorophyll *b* Zeaxanthin	Chlorophyll *b* Zeaxanthin Fucoxanthin

Chlorophyll *a*, Fucoxanthin, and Zeaxanthin

Adsorbents	Magnesia + Celite (1 to 1)	Magnesia + Celite (1 to 1)	Sugar	Sugar
Solvents	Petroleum ether + 3% ethanol	Petroleum ether + 25% acetone	Petroleum ether + 0.5% ethanol	Petroleum ether + 5% acetone + 0.25% ethanol
Adsorption sequences	Chlorophyll *a* Fucoxanthin Zeaxanthin	Chlorophyll *a* Zeaxanthin Fucoxanthin	Fucoxanthin Zeaxanthin Chlorophyll *a*	Fucoxanthin Chlorophyll *a* Zeaxanthin

Chlorophyll *a*, Violaxanthin, and Lutein

Adsorbents	Sugar	Magnesia + Celite (1 to 1)	Sugar	Sugar
Solvents	1,2-Dichloroethane	Petroleum ether + 25% acetone	Petroleum ether + 0.5% ethanol	Petroleum ether + 5% acetone + 0.25% ethanol
Adsorption sequences	Chlorophyll *a* Violaxanthin Lutein	Chlorophyll *a* Lutein Violaxanthin	Violaxanthin Lutein Chlorophyll *a*	Violaxanthin Chlorophyll *a* Lutein

Fucoxanthin, Violaxanthin, and Zeaxanthin

Adsorbents	Celite	Sugar	Magnesia + Celite (1 to 1)
Solvents	Petroleum ether + 5% acetone	Petroleum ether + 0.5% ethanol	Petroleum ether + 25% acetone
Adsorption sequences	Fucoxanthin Violaxanthin Zeaxanthin	Violaxanthin Fucoxanthin Zeaxanthin	Zeaxanthin Fucoxanthin Violaxanthin

MIXTURES WITH SEVERAL COMPONENTS

Some mixtures of three components have now been separated in three of the six possible sequences. A few mixtures of three components have been resolved in four of the six possible sequences.

Examples of three sequences obtained by adsorption of two chlorophylls and one xanthophyll are illustrated in Table I. These three sequences resulted from variation of both solvent and adsorbent. Similar sequences were observed in columns of a single adsorbent by variation of the solvent alone. Thus far it has not been possible to alter the relative adsorbabilities of chlorophylls *a*, *b*, and *d*, which are adsorbed in the order *b*, *d*, and *a*, with *a* being least adsorbed (*7*).

Examples of three adsorption sequences obtained by adsorption of chlorophyll *b* and the two xanthophylls, fucoxanthin and zeaxanthin, are reported in Table II. Mixtures of chlorophyll *a* and these two xanthophylls were resolved in four of the six possible sequences.

With mixtures of chlorophyll *a*, violaxanthin, and lutein, four adsorption sequences were likewise obtained.

Mixtures of the three xanthophylls, fucoxanthin, violaxanthin, and zeaxanthin, were readily resolvable, yielding three sequences.

Adsorption of a mixture of two chlorophylls and two xanthophylls has yielded as many as 6 of the 24 possible sequences, as illustrated in Table III. Some of the sequences reported there were altered by very slight changes in the concentration of alcohol in the solvent. In fact, different preparations of powdered sugar

Table III. Adsorption Sequences of Chlorophyll *b*, Chlorophyll *a*, Fucoxanthin, and Zeaxanthin

Adsorbents	Sugar	Sugar	Magnesia + Celite (1 to 1)	Magnesia + Celite (1 to 1)	Sugar	Celite
Solvents	Petroleum ether + 1% ethanol	Petroleum ether + 5% acetone + ca. 0.75% ethanol	Petroleum ether + 3% ethanol	Petroleum ether + 25% acetone	Petroleum ether + 5% acetone + ca. 0.5% ethanol	Petroleum ether + 5% acetone + ca. 0.5% ethanol
Adsorption sequences	Fucoxanthin Chlorophyll *b* Zeaxanthin Chlorophyll *a*	Fucoxanthin Chlorophyll *b* Chlorophyll *a* Zeaxanthin	Chlorophyll *b* Chlorophyll *a* Fucoxanthin Zeaxanthin	Chlorophyll *b* Chlorophyll *a* Zeaxanthin Fucoxanthin	Chlorophyll *b* Fucoxanthin Chlorophyll *a* Zeaxanthin	Chlorophyll *b* Fucoxanthin Zeaxanthin Chlorophyll *a*

required very careful adjustment of the alcohol concentration in order to provide the adsorption sequences shown in the table.

By variation of the amounts of alcohol and of acetone added to petroleum ether, fucoxanthin has been made to occupy four different positions relative to the bands of chlorophylls *b*, *d*, and *a* in columns of sugar. With petroleum ether containing about 1% alcohol, fucoxanthin is adsorbed above the most adsorbed green pigment, chlorophyll *b;* with petroleum ether containing about 5% acetone, fucoxanthin is adsorbed below the least adsorbed green pigment, chlorophyll *a*. At intermediate concentrations of alcohol and of acetone, fucoxanthin occupies positions between chlorophylls *b* and *d* and between *d* and *a*. These four components thus yield four sequences.

In columns of sugar and with petroleum ether containing different amounts of alcohol and acetone, fucoxanthin has been made to occupy positions above and below each of the following chlorophylls: *b*, *b'*, *a*, and *a'*. In this way five components have been made to yield five sequences.

In all the experiments leading to the results summarized in this paper, relatively few adsorbents and solvents were employed. The ease with which chlorophylls and carotenoids are decomposed by many adsorptive solids necessitated rapid work with some adsorbents and precluded the use of many others. Further investigation of the adsorption of these plant pigments will undoubtedly reveal additional adsorption sequences.

DISCUSSION

Variation of the adsorption sequence with changes of conditions in the adsorption columns demonstrates the great sensitiveness of the chromatographic adsorption method. Substances inseparable under one set of conditions may be readily separable under slightly different conditions. If substances are to be identified and named in relation to their relative adsorbabilities (*13, 15*), unusual care must be exercised in the purification of solvents and in the preparation and description of adsorbents.

In order to be regarded as "chromatographically homogeneous" (*9*) substances should be adsorbed on a variety of adsorbents from dilute solution in different solvents and solvent mixtures. Determination of the identity or nonidentity of two substances by adsorption of a mixture of them upon Tswett columns will be most conclusive when different solvents and adsorbents are employed.

Reversibility of the adsorption sequence often provides a convenient and rapid method for preparation of substances in a high state of purity, for the purity of adsorbed substances is related to the sequence. When a mixture of substances is separated in a Tswett column, each adsorbed compound may be contaminated with small, trailing portions of each less adsorbed substance (*4*). By contrast, each adsorbed substance is entirely free of the compounds which are adsorbed above it in the column. Consequently if a substance which forms an upper band can be readsorbed under conditions such that it then forms the least adsorbed band it will be free of the contaminants.

Substances adsorbed on a given adsorbent in one sequence from one solvent and in another sequence from another solvent can be made to occupy various intermediate positions by adsorption from mixtures of the two solvents. Analogously, substances adsorbed from a given solvent in one sequence in columns of one adsorbent and in another sequence in columns of a second adsorbent should be adsorbable in various intermediate positions in columns composed of mixtures of the two adsorbents.

Variation of the adsorption sequence especially among compounds of the same class indicates that the relationship between adsorbability and chemical structure is much more complex than was at first supposed. Experience with the chlorophylls and xanthophylls suggests that the relative adsorbabilities depend upon competition of solvent, solute, and adsorbent for one another. Adsorbability of some molecules is especially sensitive to the attraction between solvent and adsorbent. Adsorbability of other molecules appears to be particularly dependent upon the attractive forces between solvent and solute. With some molecules, such as indicators, change of solvent may alter the molecular structure and the reactivity of functional groups, thus affecting the adsorbability. In static systems, the summation of these effects has usually been expressed by the adsorption isotherm, but the relation between the adsorption isotherm and the chemical structure of the solute has been difficult to interpret (*1, 2*).

Recent theoretical and experimental work has shown that the rate of migration of a solute through an adsorption column is a function of the adsorption isotherm (*1, 2, 4, 5, 6, 8, 14*). It follows, therefore, that the adsorption sequence is also a function of the adsorption isotherms of the several solutes. Variation of the adsorption sequence with different conditions in the column must result from disproportionate variation of the adsorption isotherms.

As a rule the slope of the adsorption isotherm is more nearly constant at low concentrations of solutes than at higher concentrations (*2*). Consequently, the adsorption sequences should be subject to less variation and the separation of the bands from one another should be more complete when mixtures of solutes are adsorbed from dilute solution than when adsorbed from more concentrated solution.

As solutes pass through an adsorption column the bands widen so that the concentration of each solute decreases constantly. The final relative positions of the bands in a long column will therefore, be determined by the slope of the adsorption isotherms at low concentration. Theoretically, the trailing boundaries of adsorption bands provide a better basis for estimation of the adsorption sequences than do the leading boundaries (*4, 5*), but the diffuse trailing boundaries are usually more difficult to locate than the better defined leading boundaries, and they may be distorted because of the slowness with which the last traces of solute diffuse from porous particles of the adsorbent.

The resolving power of an adsorption column depends upon the properties of the adsorbent, the solvent, and the solutes. It depends in part upon the relative concentration of the solutes, upon the amount of solution added to the column, and presumably also upon the temperature. The interrelationship of these properties and conditions is so complex and the tendency of many adsorbents to combine irreversibly with the solutes is so common that the selection of solvents and adsorbents for the resolution of unknown mixtures remains largely a matter of trial and error.

Conditions that fix the sequence of solutes in columns should determine the relative adsorbabilities of these solutes in other adsorption procedures such as the fractional adsorption technique, described by Craig and co-workers (*3*), in which equal portions of the adsorbent are exposed in succession to one portion of the solution and then in turn to successive portions of the

solvent. Variation of the adsorbability may also be utilized to enhance the sensitivity and to broaden the applicability of most analytical methods based upon the phenomenon of adsorption.

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Determination of Chlorine in 2,2′-Dihydroxy-5,5′-dichlorodiphenylmethane

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Rapid determination of 2,2′-dihydroxy-5,5′-dichlorodiphenylmethane is accomplished by a modification of the U.S.P. XII method for assay of iodophthalein sodium. The compound is decomposed by alkaline permanganate solution and the chloride formed is determined by the Volhard titration or by a photometric turbidity method. The application of the method for analysis of powders, emulsions, and fabrics is of particular interest.

IN CONNECTION with work on the compound 2,2′-dihydroxy-5,5′-dichlorodiphenylmethane, an efficient mildew-proofing agent (*5*) known also as DDM, or as Compound G-4 to the textile trade, it became necessary to devise a rapid method of analysis suitable for control operations. The procedure developed depends upon the oxidation of DDM with alkaline potassium permanganate and, after acidification, reduction of excess permanganate with sodium nitrite. The liberated chloride is then determined by the Volhard (*9*) method or by the photometric measurement of the turbidity of silver chloride suspensions.

The method generally used for determination of this compound is based upon that of Willard and Thompson (*10*) and is unsatisfactory here because it is a procedure for total chlorine. Furthermore, it involves a number of steps which prolong the procedure beyond the time available for control analysis. The authors have avoided these difficulties, and have developed a method applicable to technical or pure DDM powders, solutions, or emulsions, and impregnated fabrics. The behavior of organo-iodine compounds with a method (*2*) similar to the proposed indicates that organo-halogen compounds which are alkali-soluble and easily oxidized are included in the reaction; hence the procedure is not a specific test but is admirable for control purposes.

The method here presented is similar to that of Leclercq (*4*), later modified by Butler and Burdett (*1*). The latter determined iodine in sodium tetraiodophenolphthalein by oxidation of the organic matter with alkaline potassium permanganate, and, after acidification, reduced the excess permanganate with sodium bisulfite. The iodide was then titrated with silver nitrate and the end point detected with an adsorption indicator. Excellent results were obtained which were checked by the Pregl (*6*) combustion method on a semimicro scale. The Butler-Burdett method is now one of the official U.S.P. XII (*8*) procedures for the determination of iodine in organic pharmaceuticals.

The determination of iodine in organic compounds by an acid permanganate oxidation is well known. Seeker and Mathewson (*7*) used an acid permanganate oxidation to determine the iodine content of erythrosine. Of more recent date is the work of Clark and Jones (*2*) who employed both an acid and alkaline permanganate method for the determination of iodine in organic compounds soluble in acids or bases. To the knowledge of the authors, the alkaline permanganate oxidation method has not yet been applied to the determination of chlorine in organic compounds. No claim is made of its general applicability to organic compounds containing chlorine, but it has been successfully applied to the estimation of DDM.

Table I. Analysis of Purified DDM

Method	Chlorine	Deviation from Theory
	%	%
Proposed	26.12	−0.24
Pregl	26.46	+0.10

PROCEDURE FOR DDM POWDER

Place approximately 0.2 gram of DDM powder, accurately weighed, into a 500-ml. Erlenmeyer flask and dissolve in 15 ml. of 10% sodium hydroxide. Add 35 ml. of saturated potassium permanganate; the characteristic purple color of the permanganate should persist. If the mixture is green, add more saturated potassium permanganate. Add a few glass beads to control bumping and boil the mixture gently for 10 minutes. Cool under the tap to room temperature and add 75 ml. of distilled water followed by 10 ml. of concentrated nitric acid. Reduce the excess permanganate immediately with 10% sodium nitrite, preferably added from a buret. Add 25 ml. of 0.1 *N* silver nitrate and titrate the excess with 0.1 *N* ammonium thiocyanate, using 2 ml. of ferric alum as the indicator.

Perform a blank determination with the same quantities of the same reagents and in the same manner.

DISCUSSION OF METHOD

The proposed method was checked by analysis of both technical and pure DDM. Purified DDM, prepared from the technical grade by three crystallizations from ethylene dichloride followed by a single crystallization from distilled water, was analyzed by the proposed method and checked by a Pregl microcombustion analysis (Table I). From the good agreement obtained with these independent methods, it is apparent that oxidation of the DDM is complete with alkaline permanganate, and that the method is more than sufficiently accurate for control procedures.

Table II. Analysis of Technical DDM

Sample	Per Cent DDM Proposed method	Pregl method
A	94.2	99.6
B	93.9	96.4

Table III. Analysis of DDM on Fabric

Sample No.	Per Cent of DDM on Fabric Gottlieb-Marsh method	Silver chloride turbidity method
1	0.89	0.84
		0.80
2	1.09	1.04
		1.05
3	1.51	1.56
		1.55
4	1.72	1.68
		1.64

The proposed method has been applied to the analyses of technical DDM samples. An inspection of the results in Table II shows that analysis of technical DDM by total halogen determination may lead to erroneous conclusions as to the DDM content. The lower DDM values obtained by the proposed method compared with those obtained by Pregl microcombustion analysis would indicate the presence in technical DDM of organic halogen compounds which were not oxidized by the alkaline permanganate method. A check of these technical DDM samples revealed a negligible amount of inorganic halogen present.

ANALYSIS OF EMULSIONS

The analysis of emulsions containing DDM, frequently used to impart water-repellency as well as mildew-proofness to fabrics, requires little change in the procedure described previously for DDM powder. For the purpose of choosing a sample weight or aliquot volume, 0.1 gram of DDM requires 7.43 ml. of 0.1 *N* silver nitrate. The wax used in these emulsions presents no difficulty, since it readily separates to the surface during oxidation and does not interfere with the final titration.

ANALYSIS OF FABRICS

Because of the small quantity of DDM generally applied to fabrics, the chloride content of the oxidized extract does not permit convenient titration. However, the liberated chloride is readily determined by photometric methods. The acidity of the solution following addition of sodium nitrite is adjusted so that the silver chloride is maintained in suspension. The turbidity of this suspension is then measured with a suitable photometer—a Fisher electrophotometer was employed in the work presented here—and the DDM content determined by reference to a standard calibration curve.

Solutions. Nitric acid, 1 to 4. Dilute 50 ml. of concentrated nitric acid, sp. gr. 1.42, with 150 ml. of distilled water.

Sodium carbonate, 0.025%. Dissolve 0.5 gram of anhydrous sodium carbonate in 2 liters of distilled water.

Sodium hydroxide, 2.5%. Dissolve 5 grams of sodium hydroxide in 195 ml. of distilled water.

Phenolphthalein indicator, 0.5%. Dissolve 0.5 gram of phenolphthalein in 100 ml. of 95% ethyl alcohol.

Standard Curve. Since the logarithm of the transmittance of silver chloride suspensions is an inverse and very nearly linear function of the turbidity, four determinations are adequate to establish the curve. To illustrate this point, 2, 4, 6, 10, and 15 mg. of DDM in a final volume of 200 ml. gave respective photometric logarithmic readings (corrected for reagent blank) of 9.8, 18.0, 28.1, 48.1, and 74. Establish the curve by first dissolving 500 mg. of DDM in sufficient 2.5% sodium hydroxide to make 100 ml. of solution. Use suitable aliquots of this solution, containing 5 mg. of DDM per ml., to cover the range of 2 to 15 mg. The procedure followed is exactly as described below for the fabric extract.

Fabric Analysis. Weigh a 2-gram sample of the very finely cut fabric to the nearest milligram and place in a 150-ml. beaker. Extract three times with successive 50-ml. portions of 0.025% sodium carbonate by boiling gently for 5 minutes and decanting the hot solution into a 200-ml. Pyrex volumetric flask. Wash the extracted fabric twice with 15-ml. portions of 0.025% sodium carbonate and combine with the extract solution. Dilute to the mark with 0.025% sodium carbonate after the flask has cooled to room temperature. Filter about 50 ml. through a dry filter. Then follow the oxidation procedure for analysis of DDM powder with these exceptions: Use a 25-ml. aliquot of the filtrate; reduce the volume of saturated potassium permanganate to 25 ml.; omit the dilution with water after oxidation. Following reduction of the excess permanganate with 10% sodium nitrite, add a few drops of phenolphthalein indicator and then sufficient 10% sodium hydroxide to produce a faint pink color. Acidify with 1 ml. of 1 to 4 nitric acid and transfer to a 200-ml. volumetric flask. Cool to room temperature, add 4 ml. of 0.1 *N* silver nitrate, dilute to the mark with distilled water, and invert the flask several times. Let stand 10 minutes and read with the Fisher electrophotometer using a 525 filter.

With the above procedure it is necessary to establish two blank values—extract blank and reagent blank. For the extract blank, pipet a 25-ml. aliquot of the filtrate into a 200-ml. volumetric flask, add 125 ml. of 0.025% sodium carbonate, 10 ml. of 1 to 4 nitric acid, and 4 ml. of 0.1 *N* silver nitrate, and dilute to the mark. Invert the flask several times and read after 10 minutes. This extract blank, representing inorganic chloride, is frequently negligible. For the reagent blank, follow the procedure described above, omitting the 25-ml. extract aliquot. This reagent blank value need be determined only once for a set of reagents.

The extract procedure above described is essentially the same as that developed by Gottlieb and Marsh (*3*) for the determination of DDM by the color reaction with 4-aminoantipyrine in the presence of potassium ferricyanide and dilute sodium carbonate. Table III gives the comparative results obtained from analysis of four samples of fabric by the Gottlieb-Marsh colorimetric and silver chloride turbidity methods.

CONCLUSIONS

The alkaline permanganate procedure used successfully for the analysis of certain organic iodine compounds has been adapted for the routine analysis of DDM. Analysis of purified DDM indicates the reaction is complete, as substantiated by a Pregl microcombustion analysis. The alkaline permanganate method appears to be of particular value in the analysis of technical DDM which has organic chlorine-containing impurities. Analysis of such technical material indicates that these impurities are not as susceptible to oxidation by the proposed method as by the Pregl microcombustion total halogen method. Of further particular interest is the applicability of the described method for analysis of powders, emulsions, and fabrics containing DDM.

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Viscosities of Pure Hydrocarbons

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THE purpose of this paper is to record the viscosities of pure hydrocarbons.

The viscosities were determined in several viscometers of the pe shown as Figure 1, which is but a slight modification of the pe previously recommended for nonviscous materials (*4*).

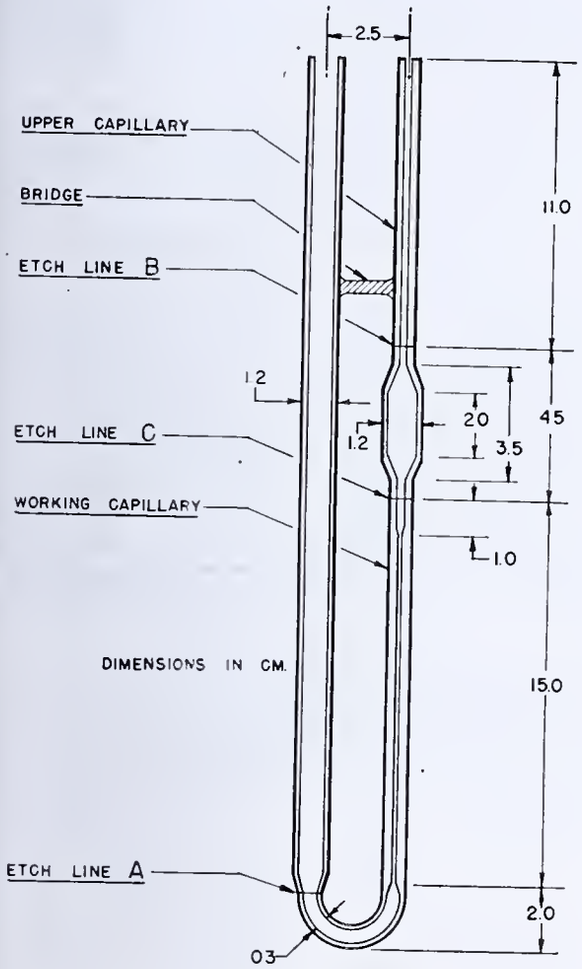

Figure 1. Type C Viscometer

er this work was finished it was found that the viscometer wn as Figure 2 is faster to use and just as accurate, and it is ng used in extending this work. For viscous liquids above) centipoises it is more accurate because the larger diameter and er reservoir reduces the effect of drainage on the driving fluid d. It is used in the manner described previously (*4*). For low viscosity data reported here, this drainage effect is below 5%.

The most troublesome source of error in the viscosity range ow 2 centipoises is the so-called "kinetic energy" error. This kept small by so designing the viscometers that velocities e kept low enough to prevent this correction from exceeding %. As the probable error in making this correction is of the er of ±20%, a net error of 0.04% may occur from this source. ier sources of error in viscosity measurements have been dis- sed in detail (*4*).

The type C viscometer shown as Figure 1 is loaded as pre- usly described (*4*). A slight excess of 0.2 or 0.3 cc. is then added to the instrument which is placed in an accurately controlled constant-temperature bath. The 0° C. bath was controlled to within ± 0.05° C. while the 20° C. and 40° C. baths were controlled to within ±0.01° C. When bath temperature is attained, slight pressure is applied to the wide arm of the viscometer and the bottom of the meniscus is brought to etched line *A*. The slight excess liquid is then wiped from the capillary arm. The excess so removed will be somewhat different from the excess charged, because the volume of liquid will change in going from room temperature to the test temperature of the bath. This simple technique allows the same viscometer constant to be used over a wide temperature range, although other methods of making the necessary slight corrections in the viscometer constant for each temperature are satisfactory. The viscometers were calibrated with distilled water at 20° C. and checked by hydrocarbons which had been standardized in master viscometers (*3*). The viscosity of the test sample is obtained by multiplying the efflux time in seconds (the efflux volume timed is that between lines *B* and *C*) by the viscometer constant. This yields kinematic viscosity in centistokes, which in turn must be multiplied by density in order to obtain absolute viscosity in centipoises.

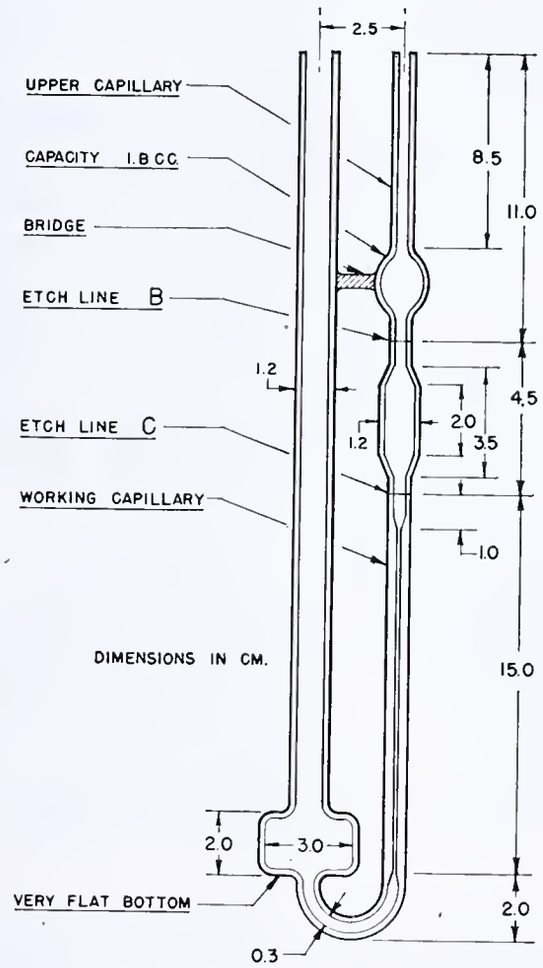

Figure 2. Type E Viscometer

All samples were tested with two separate viscometers and stop watches. The stop watches were spring-wound and tested against a large electric clock operated by constant-frequency constant-voltage power. The spring-wound stop watches were found more reliable than several types of electric stop clocks which operate by a magnetic clutch engaging and disengaging a constant-speed rotor. In these the clutch frequently slips and sometimes fails to make a sharp disengagement, so that the clock drifts past the true stopping point. The correction necessary on the spring-wound stop watches employed never exceeded 0.05%.

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Table I. Viscosities of Octanes

(Listed in decreasing value at 20° C.)

Hydrocarbon	Density			Kinematic Viscosity			Absolute Viscosity		
	0° C.	20° C.	40° C.	0° C.	20° C.	40° C.	0° C.	20° C.	40° C.
	Gram per cc.			*Centistokes, $cm^2. \times sec.^{-1} \times 10^{-2}$*			*Centipoise, $g. \times cm.^{-1} \times sec.^{-1} \times 10^{-2}$*		
2,2,3,3-Tetramethylbutane	Solid	Solid	Solid	Solid	Solid	Solid	Solid	Solid	Solid
2,3,3-Trimethylpentane	0.741	0.726	0.711	1.227	0.933	0.739	0.909	0.677	0.525
2,2,3-Trimethylpentane	0.731	0.715	0.699	1.074	0.836	0.674	0.785	0.598	0.471
3-Methyl-3-ethylpentane	0.742	0.727	0.712	1.034	0.799	0.642	0.768	0.581	0.457
2,3,4-Trimethylpentane	0.734	0.719	0.703	0.969	0.764	0.624	0.712	0.549	0.438
n-Octane	0.718	0.712	0.686	0.985	0.771	0.626	0.707	0.542	0.429
2,2-Dimethylhexane	0.711	0.695	0.679	0.975	0.758	0.611	0.694	0.527	0.414
2,2,4-Trimethylpentane	0.707	0.691	0.675	0.917	0.738	0.598	0.649	0.504	0.403
2-Methylheptane	0.713	0.698	0.681	0.909	0.717	0.586	0.648	0.500	0.399
3,3-Dimethylhexane	0.726	0.710	0.694	0.890	0.703	0.575	0.645	0.499	0.399
2,3-Dimethylhexane	0.729	0.713	0.697	0.881	0.698	0.571	0.643	0.497	0.398
3,4-Dimethylhexane	0.734	0.718	0.703	0.862	0.686	0.564	0.633	0.493	0.396
3-Methylheptane	0.721	0.705	0.689	0.886	0.697	0.570	0.639	0.492	0.393
2-Methyl-3-ethylpentane	0.735	0.719	0.702	0.856	0.678	0.556	0.628	0.488	0.390
2,5-Dimethylhexane	0.710	0.693	0.677	0.883	0.698	0.571	0.626	0.484	0.386
4-Methylheptane	0.720	0.704	0.689	0.829	0.659	0.542	0.597	0.464	0.373
2,4-Dimethylhexane	0.716	0.700	0.683	0.824	0.658	0.542	0.590	0.461	0.370
3-Ethylhexane	0.729	0.714	0.697	0.799	0.635	0.523	0.583	0.453	0.364

Table II. Viscosities of Some 5-, 6-, and 7-Carbon Hydrocarbons

Hydrocarbon	Density			Kinematic Viscosity			Absolute Viscosity		
	0° C.	20° C.	40° C.	0° C.	20° C.	40° C.	0° C.	20° C.	40° C.
	Gram per cc.			*Centistokes*			*Centipoises*		
n-Pentane	0.644	0.626	Vapor	0.426	0.362	Vapor	0.274	0.227	Vapor
Isopentane	0.638	0.619	Vapor	0.435	0.363	Vapor	0.278	9.225	Vapor
Cyclopentane	0.764	0.745	0.726	0.728	0.590	0.489	0.555	0.440	0.355
n-Hexane	0.677	0.659	0.641	0.564	0.468	0.397	0.382	0.308	0.254
2-Methylpentane	0.671	0.653	0.635	0.523	0.437	0.372	0.351	0.286	0.237
3-Methylpentane	0.681	0.664	0.646	0.544	0.451	0.382	0.371	0.299	0.247
2,3-Dimethylbutane	0.679	0.661	0.642	0.626	0.508	0.422	0.425	0.336	0.271
Methylcyclopentane	0.766	0.748	0.730	0.848	0.677	0.555	0.650	0.507	0.405
Cyclohexane	Solid	0.778	0.759	Solid	1.259	0.926	Solid	0.980	0.703
Cyclohexene	0.831	0.813	0.794	1.066	0.816	0.653	0.886	0.664	0.518
Benzene	Solid	0.879	0.857	Solid	0.739	0.573	Solid	0.649	0.492
n-Heptane	0.700	0.683	0.666	0.745	0.602	0.499	0.521	0.411	0.333
2,2,3-Trimethylbutane	0.707	0.690	0.673	1.141	0.853	0.668	0.806	0.589	0.449
Ethylcyclopentane	0.783	0.766	0.749	0.928	0.740	0.609	0.726	0.567	0.456
Methylcyclohexane	0.787	0.769	0.753	1.263	0.954	0.750	0.993	0.734	0.564
Toluene	0.883	0.865	0.848	0.878	0.676	0.549	0.774	0.585	0.465

Table III. Viscosities of Some 8-, 9-, and 10-Carbon Hydrocarbons

Hydrocarbon	Density			Kinematic Viscosity			Absolute Viscosity		
	0° C.	20° C.	40° C.	0° C.	20° C.	40° C.	0° C.	20° C.	40° C.
	Grams per cc.			*Centistokes*			*Centipoises*		
o-Xylene	0.897	0.880	0.862	1.240	0.922	0.723	1.111	0.811	0.624
m-Xylene	0.881	0.864	0.847	0.916	0.714	0.581	0.807	0.617	0.492
p-Xylene	Solid	0.861	0.843	Solid	0.748	0.602	Solid	0.643	0.507
Ethylbenzene	0.886	0.868	0.851	1.010	0.781	0.631	0.895	0.678	0.536
Ethylcyclohexane	0.804	0.788	0.772	1.420	1.068	0.842	1.142	0.842	0.651
cis-1,2-Dimethylcyclohexane	0.812	0.796	0.780	1.988	1.399	1.048	1.615	1.114	0.818
trans-1,2-Dimethylcyclohexane	0.791	0.776	0.760	1.404	1.053	0.825	1.111	0.817	0.627
cis-1,3-Dimethylcyclohexane	0.802	0.785	0.769	1.501	1.103	0.850	1.203	0.866	0.654
trans-1,3-Dimethylcyclohexane	0.782	0.766	0.750	1.063	0.825	0.667	0.831	0.631	0.500
cis-1,4-Dimethylcyclohexane	0.799	0.783	0.766	1.531	1.119	0.861	1.224	0.875	0.659
trans-1,4-Dimethylcyclohexane	0.779	0.762	0.746	1.222	0.926	0.733	0.951	0.706	0.547
n-Propylcyclopentane	0.792	0.776	0.756	1.123	0.877	0.711	0.889	0.681	0.540
Isopropylcyclopentane	0.792	0.776	0.761	1.162	0.909	0.735	0.920	0.706	0.559
1,1,3-Trimethylcyclohexane	0.794	0.778	0.763	1.510	1.120	0.871	1.199	0.873	0.664
n-Propylcyclohexane	0.809	0.793	0.778	1.744	1.268	0.976	1.410	1.005	0.759
Isopropylcyclohexane	0.817	0.803	0.786	1.880	1.369	1.049	1.535	1.097	0.824
n-Propylbenzene	0.878	0.862	0.845	1.348	0.995	0.778	1.183	0.857	0.657
Isopropylbenzene	0.879	0.862	0.845	1.226	0.917	0.725	1.077	0.790	0.613
2-Methylnonane	0.742	0.727	0.712	1.644	1.199	0.925	1.220	0.872	0.659
3-Methylnonane	0.747	0.733	0.718	1.582	1.159	0.898	1.182	0.849	0.645
4-Methylnonane	0.747	0.732	0.716	1.498	1.100	0.854	1.118	0.804	0.612
5-Methylnonane	0.747	0.732	0.717	1.526	1.116	0.866	1.140	0.816	0.620

The viscosities obtained in the separate viscometers were in disagreement by less than 0.1%. Efflux times were maintained above 200 seconds in order to make accurate time measurements possible as well as keep the kinetic energy error low. The accuracy of the viscosity data is dependent upon the accuracy to which the viscosity of water at 20° C. is known, since it was employed as the primary calibrating liquid. A value of 1.007 centistokes was used, as recommended by the American Society of Testing Materials (*1*). Because of the great difficulties in determining absolute viscosities, the error inherent in the 1.007 value may be of the order of ±0.5%.

However, the precision of the data recorded here is ±0.1%. Therefore, while absolute viscosities may be in error by as much as 0.5%, the comparative viscosities are in error by only 0.1%

and one compound may compared with another that degree of precision.

The viscosities of eighteen isomeric octanes listed in Table I accor to decreasing absolute cosity at 20° C. Many the compounds have wi different viscosity temp ture coefficients; thus 20° C. the absolute cosity of 3,4-dimethylhex is higher than that of methylheptane, but the rev is true at 0° C.

From a study of Ta II and III it is seen in general cyclic compou have higher viscosities a given temperature than pa finic compounds with same number of car atoms. The cis compou listed have higher viscosi than the trans, and in gen the alkylcyclohexanes are m viscous than the correspo ing alkylbenzenes.

Andrade and others *6*) have correlated visco with temperature by me of the following equation:

$$\eta = A(e)^{B/RT}$$

where η = viscosity in ce poises
R = gas constant
T = absolute temp ture
e = natural logarit base
A and B = consta for a given c pound

With the data listed Table I the values of A B were determined for 2, trimethylpentane, and cosities at other temperatu were then calculated. Ev (*5*) measured the viscosit of this compound at seve temperatures; hence it possible to compare the calculated values with measured val (Table IV).

Table IV. Viscosity of 2,2,4-Trimethylpentane

Temperature ° C.	Observed Viscosity, Evans *Centipoise*	Calculated Viscosity, Andrade Equation *Centipoise*
5	0.608	0.607
15	0.534	0.535
30	0.447	0.449
45	0.380	0.383
60	0.327	0.331
75	0.284	0.290
90	0.248	0.257

ACKNOWLEDGMENT

The samples of A.P.I.-N.B.S. hydrocarbons were made available by the American Petroleum Institute and the National ureau of Standards through A.P.I. Research Project 44 on the ollection, Analysis, and Calculation of Data on the Properties Hydrocarbons. The samples were purified at the National ureau of Standards by A.P.I. Research Project 6 on the nalysis, Purification, and Properties of Hydrocarbons, under ie supervision of Frederick D. Rossini, from material supplied y the following laboratories: *o*-xylene, Standard Oil Development Company, Elizabeth, N. J., through the courtesy of 'illiam J. Sweeney; 2,2-dimethylbutane, A.P.I. Research roject 6 at the National Bureau of Standards; the four methyl-)nanes by the Ethyl Corporation through the courtesy of eorge Calingaert; all others by A.P.I. Research Project 45 on the Synthesis and Properties of Hydrocarbons of Low Molecular Weight, at the Ohio State University under the supervision of Cecil E. Boord.

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Part of a thesis submitted by J. M. Geist in partial fulfillment of the requirements for the degree of master of science in chemical engineering at The Pennsylvania State College.

Chromatographic Resolution of the Quinone Oximes

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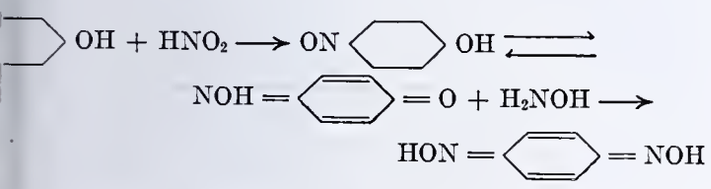
chromatographic procedure is described for the separation of *p*-nzoquinone monoxime from *p*-benzoquinone dioxime and prod-ts of side reactions which take place during the nitrosation of enol and oximation. The sample is dissolved in acetone and sorbed on a column of activated alumina. The monoxime forms a lliant green band which is removed from the column and eluted :h aqueous sodium hydroxide. The concentration is determined spectrophotometric mea:urements at 363 and 399 mμ. The omatographic resolution and optical properties of the ortho iso-rs are also discussed.

)OTH *p*-benzoquinone dioxime and its derivatives have been used for the nonsulfur vulcanization of rubber (*8*), and are current interest for the vulcanization of GR-I. The parent npound is usually formed by the nitrosation of phenol (*17*), lowed by oximation (*12*).



)uring the course of work on the preparation of this material vas of interest to follow the rate of oximation, and determine concentration of unreacted monoxime in the final product. \s this substance is tautomeric with *p*-nitrosophenol, a method cribed by Clauser (*5*) for the determination of the nitroso up by reaction with phenylhydrazine and measurement of lved nitrogen was investigated. However, under these con-ons a large volume of gas is obtained from the dioxime. Both npounds are reduced by potassium iodide in hydrochloric acid ition (*6*), but the reduction of the monoxime proceeds more idly. This suggested the possibility of a polarographic anal-. At a pH of 7.0 in a phosphate buffer, the half-wave poten-of the monoxime was found to be 0.15 volt more positive than t of the dioxime, and on solutions of the pure compounds, the usion currents are well defined. However, an unresolved ve is usually obtained on the crude product, owing to an un-orable concentration ratio and the presence of other electro-ucible materials in the mixture.

-Benzoquinone monoxime is readily soluble in dilute sodium troxide with the formation of the sodium salt, which is be-ed to exist largely in the ionized quinone oxime form (*1*, *10*). e principal absorption maximum of the ion is at 399 mμ (Figure 1), while that of the neutral molecule in acid solution is at 301.5 mμ. Anderson and Yanke (*1*) point out that the absorption spectrum of the system is sensitive to changes in hydrogen-ion concentration between pH 3 and 7 and is independent of this factor in more strongly acid or alkaline solutions. In order to ensure reproducibility of the extinction coefficients, and provide a

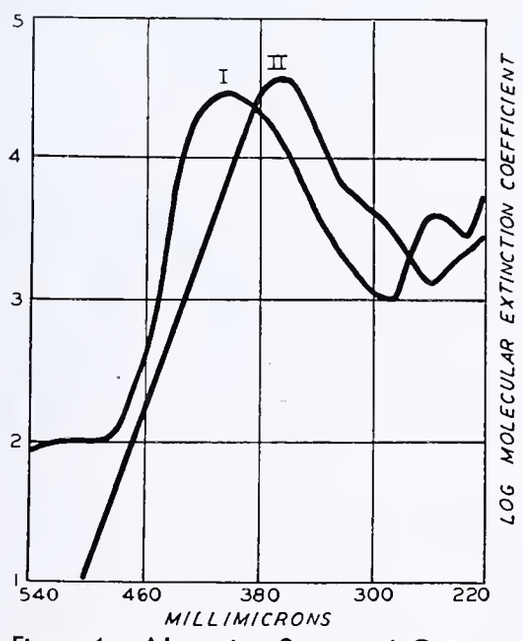


Figure 1. Absorption Spectra of Quinone Oximes in 0.1 *N* Aqueous Sodium Hydroxide Solution

I. *p*-Benzoquinone monoxime
II. *p*-Benzoquinone dioxime

medium in which the product is sufficiently soluble, the quantitative measurements described in this paper were made in 0.1 *N* sodium hydroxide solution. Quinone dioxime behaves in an analogous manner. The principal maximum attributable to the free ion is at 363 mμ (Figure 1), while that of the neutral molecule occurs at 317 mμ (Figure 2). As both compounds obey the Beer-Lambert law within experimental error, a direct calculation of the composition of a binary mixture is possible from optical readings made at 363 and 399 mμ on solutions of the sodium salts, providing no interfering substances are present and the concentration ratio is favorable. As these conditions cannot be predicted, and in general are not satisfied, it is necessary to remove the products

of side reactions which take place during the nitrosation of phenol and concentrate the monoxime at the expense of the dioxime without loss of the former.

When a solution of the crude oximation product in acetone is percolated through a column containing activated alumina, the constituents of the mixture adsorb in easily distinguishable zones which can be further resolved by washing the column with a more polar solvent. The monoxime forms a green zone, while the dioxime forms a brilliant yellow zone. The schematic diagram (Figure 3) illustrates the separations normally obtained. Band I is dark brown in color, and contains an amorphous material of indefinite composition produced by resinification of the monoxime (*17*). Band II contains *p*-benzoquinone monoxime, and Band III, *p*-benzoquinone dioxime. In the lower section of the column, narrow blue, red, and yellow bands appear which are completely removed from the column during development with solvent. The initial fraction of the percolate is orange yellow in color, and contains substances not readily adsorbed from acetone by alumina. On evaporation of the solvent and readsorption from petroleum ether, these materials can be further resolved.

The green zone containing quinone monoxime initially forms directly below the brown resinous material and is not readily separated from it by continued washing. Complete resolution is achieved by treatment of the column with a dilute solution of acetic acid in acetone. The acid is adsorbed less strongly than the resinous material, and more strongly than the monoxime which results in the formation of a colorless zone between the two materials. The development of the column is completed by washing with 300 ml. of a 5% solution of methanol in acetone. The effect of this solvent is to wash the lower zones and part of the dioxime into the percolate, and improve the separation of the oximes. At the end of this treatment the green zone contains all of the monoxime but is contaminated with 20 to 35% dioxime which is not readily removed by further washing. As a spectrophotometric correction can be made for residual dioxime, a complete separation is unnecessary. The analytical values in Table I show that after 300 ml. of solvent have been used the results are independent of the volume of solvent used in developing the chromatogram and the purity of the zone.

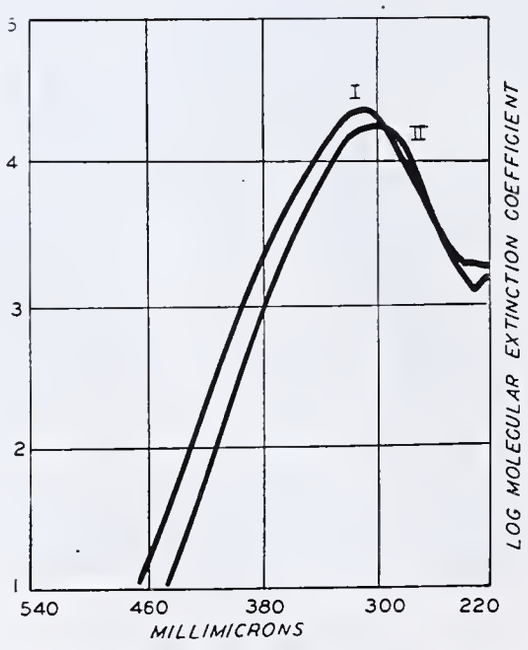


Figure 2. Absorption Spectra of Quinone Oximes in 0.1 *N* Aqueous Sulfuric Acid Solutions

I. *p*-Benzoquinone monoxime
II. *p*-Benzoquinone dioxime

The analysis is completed by separating the adsorbent containing monoxime from the rest of the column and eluting it with aqueous sodium hydroxide. A yellow-brown solution is obtained on which the monoxime content is determined by absorption measurements at 399 and 363 mμ. In making the analysis as outlined, low results are obtained owing to incomplete elution of the material from the adsorbent. Experiments made on the pure compound indicate that the average recovery is 95.0 ± 0.8%. When this factor is incorporated into the calculations, results close to theory are obtained.

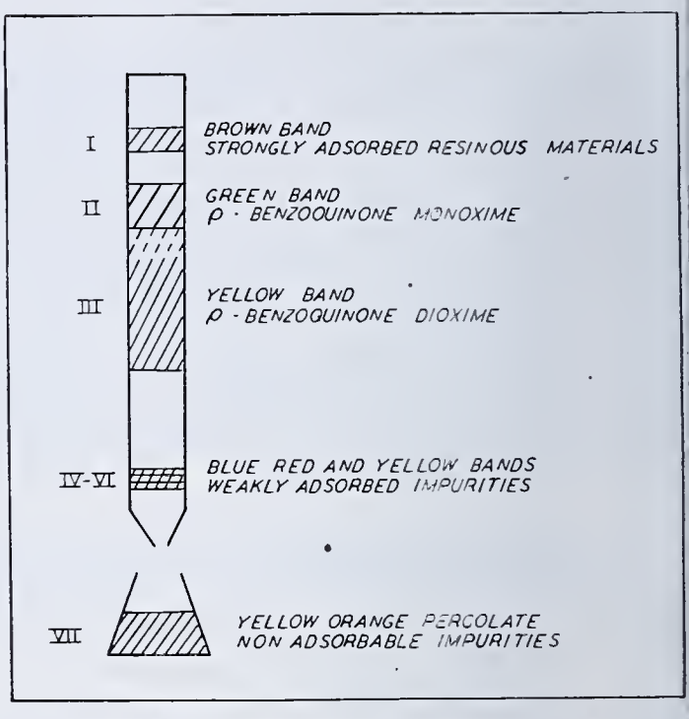


Figure 3. Chromatographic Resolution of Crude *p*-Benzoquinone Dioxime

Table I. Effect of Volume of Solvent Used in Development Chromatogram on Results of Monoxime Analysis and Purity of Zo

Solvent	Monoxime Found	Dioxime in Monoxime Zone
Ml.	%	%
0	4.9	72
100	5.5	28
200	5.4	34
300	5.6	23
500	5.6	25
800	5.6	35
1000	5.6	20

Table II. Analytical Constants

Compound	*K* at 399 mμ	Slit Setting *Mm.*	*K* at 363 mμ	Slit Sett *Mm.*
p-Benzoquinone monoxime	224	0.10	109	0.15
p-Benzoquinone dioxime	283	0.10	65.7	0.15

$$\text{Per cent monoxime} = \frac{100\ V\ (K'_{\lambda_2}D_{\lambda_1} - K'_{\lambda_1}D_{\lambda_2})}{GF\ (K'_{\lambda_2}K_{\lambda_1} - K'_{\lambda_1}K_{\lambda_2})}$$

where D_{λ_1} and D_{λ_2} are the measured optical densities at 399 a 363 mμ, G is the sample weight, V is the volume in liters to wh the solution is diluted, K'_{λ_1} and K_{λ_2} are the specific extinction efficients of the monoxime at 399 and 363 mμ, K'_{λ_1} and K'_{λ_2} the specific extinction coefficients of the dioxime at 399 and mμ, and F is the decimal per cent monoxime recoverable af adsorption on alumina.

This equation is a modification of the type generally used in spectrophotometric analysis of binary mixtures where the sorption bands overlap. Details concerning the derivation given by Ashley (*3*).

Spectrophotometric constants determined on solutions of pure compounds in 0.1 *N* sodium hydroxide are shown in Ta II. The optical measurements were made with a Beckm Model DU spectrophotometer in 1-cm. quartz cells, using tungsten lamp and a red purple filter. Both compounds ob the Beer-Lambert law within experimental error at concent tions corresponding to a density range of 0.4 to 1.0.

APPARATUS

Adsorption columns are conveniently made from 35-cm. leng of 18-mm. outside diameter Pyrex tubing constricted to internal diameter of about 0.5 cm. about 10 cm. from the e

Table III. Analyses of Synthetic Mixtures

Monoxime Added %	Monoxime Found %	Deviation %
5.5	5.7	+0.2
4.3	3.9	−0.4
4.5	4.3	−0.2
4.6	4.4	−0.2
6.5	6.7	+0.2
4.9	5.5	+0.6
5.8	5.5	−0.3
6.0	6.2	+0.2
7.8	8.0	+0.2
4.8	5.0	+0.2
4.8	4.8	0.0
7.0	6.9	−0.1
Av. 5.54	5.58	±0.23

Table IV. Analysis of Crude Reaction Products

Monoxime, %	4.9	5.6	3.5	4.8	4.4
Dioxime, %	90.7	84.5	79.4	77.8	91.3
Resinous material, %	2.6	4.5	14.6	10.9	3.1
Inorganic, %	0.8	2.9	1.6	1.1	0.1
Total	99.0	97.5	99.1	94.6	98.9

The shorter end is connected to a suction flask and the constriction plugged with glass wool. The column is filled to a height of 15 cm. with a mixture of 90 parts by weight of grade F-1 — 80-mesh activated alumina (Aluminum Company of America) and 10 parts of Hyflo Super-Cel (Johns Manville Company) which is included to increase the percolation rate. The adsorbent is added to the column in 2- to 3-cm. portions and tamped firmly into place with a wooden dowel. Solvent is delivered continuously to the head of the column from a 500-ml. separatory funnel.

REAGENTS

Anhydrous analytical reagent grade solvents are used for the preparation of the chromatogram. The column is developed with a 1% by volume solution of acetic acid in acetone, and a 5% solution of anhydrous methanol in acetone. The adsorbent is eluted with aqueous *N* sodium hydroxide.

PROCEDURE

A 1-gram sample is dissolved in 100 ml. of acetone under reflux and filtered through a tared Selas crucible to remove acetone-insoluble resin and inorganic constituents. If desired, the amounts of these materials can be determined by the usual gravimetric procedures. The filtrate is reduced to half its original volume on a steam bath and after cooling percolated through a column containing the adsorbent. The column is then washed with 10 ml. of acetic acid solution, followed by 300 ml. of methanol in acetone. The top brown layer is removed with a spatula and discarded. The green segment is transferred quantitatively to a 250-ml. beaker and eluted with successive portions of sodium hydroxide. A total volume of about 100 ml. is required. The separation of the yellow zone need not be complete, as a correction for residual dioxime is made in the calculations. After filtration, the elutriate is diluted to 1 liter with distilled water and then rediluted to a concentration at which optimum optical density readings can be obtained. The measurements are made at 399 and 363 mμ with a Beckman spectrophotometer.

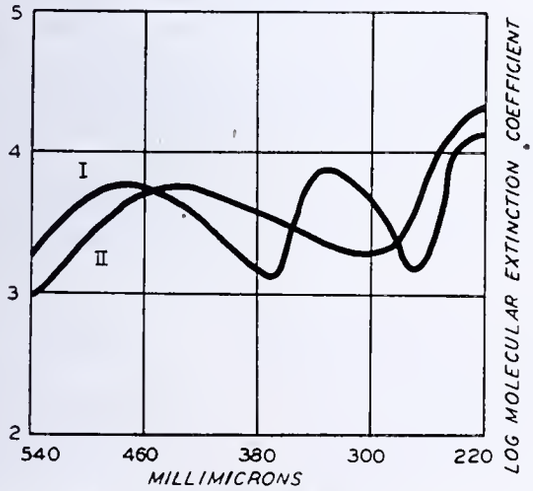


Figure 4. Absorption Spectra of Quinone Oximes in 0.1 *N* Aqueous Sodium Hydroxide Solution

I. *o*-Benzoquinone monoxime
II. *o*-Benzoquinone dioxime

PRECISION AND ACCURACY

The appearance of the adsorption column is subject to variation, depending on the composition of the sample and differences in the techniques used in preparing and developing the chromatograms. Impurities in the solvents and changes in the adsorptive capacity of the alumina may alter the widths of the bands formed from equal amounts of monoxime. Although a measurement of the band width will not give a quantitative estimate of the concentration, the appearance of a green zone provides direct visual evidence of the presence of the monoxime. A band width of 2 to 6 mm. is obtained on samples containing 10 mg. of this compound. Thus, the method is sufficiently sensitive to establish the presence of this substance in mixtures in which it occurs to the extent of less than 1%.

In order to evaluate the accuracy of the method under the conditions normally encountered in the analysis of crude reaction products, weighted quantities of the pure monoxime were added to a sample on which a preliminary analysis of 5.6% was obtained. This value was then subtracted from the analytically determined results. This information is shown in Table III. The average deviation is ±0.23%, while the average of the analytically determined values is 0.04% higher than theory. The precision of the method as determined by the analysis of 64 samples in duplicate by a routine analyst is ±3.6% of the material present. This series included samples containing from 2 to 55% quinone monoxime.

The use of this method for the analysis of crude reaction products is illustrated by the data in Table IV. The quinone dioxime content of the materials was determined by a gravimetric procedure based on its oxidation to polymeric "*p*-dinitrosobenzene" (*13*) with potassium ferricyanide in a carbonate buffer. The materials in bands IV to VI and the percolate are not included in the analyses. These values are not representative of the yields obtained on nitrosation and oximation, as the determinations were made on samples which were partly purified by separation from the mother liquor.

While the results obtained by the chromatographic procedure are not comparable in precision to those obtained by direct spectrophotometric methods under favorable conditions, in this application the removal of interfering impurities and the concentration of the minor constituent permits a reasonably accurate analysis which would otherwise be impossible to obtain.

DISCUSSION

The para isomer is the principal reaction product formed in the nitrosation of phenol. However, Veibel (*17*) has shown that as much as 10% of the *o*-nitrosophenol may be formed under certain conditions. As this compound is tautomeric, oximation would result in the presence of *o*-benzoquinone dioxime in the crude product. In order to investigate the possibility of interference from this source, the chromatographic behavior and optical properties of the ortho isomers were investigated. The experimental data are outlined in Table V.

Both ortho isomers adsorb above *p*-benzoquinone dioxime, and if present, can be mechanically removed from the column before elution. In the reaction products examined, these bands were not evident, probably owing to loss by solubility of the *o*-monoxime in the mother liquor.

Table V. Chromatographic and Spectrophotometric Properties of Quinone Oximes

	o-Monoxime	*o*-Dioxime	*p*-Monoxime	*p*-Dioxime
Position of band	I	II	III	IV
Color of band	Red	Orange	Green	Yellow
$\lambda_{max.}$ (ion) (mμ)	470	433.5	399	363
$\epsilon_{max.}$ (ion)	6,130	5,600	27,570	39,010
$\lambda_{max.}$ (molecule) (mμ)	400	398	301.5	317
$\epsilon_{max.}$ (molecule)	1,110	5,720	16,820	23,300
Dipole moment ($\mu \times 10^{18}$)	...	3.84	4.72	2.37

The isomeric dioximes are adsorbed in the order of their dipole moments, the ortho isomer with a moment of 3.84 *D* (*11*) forming the upper orange band, and the para isomer with a moment of 2.37 *D* forming the lower yellow band. This behavior is analogous to that of the position isomers of benzene, and to the cis-trans isomers of azobenzene in which the compound with the higher dipole moment is usually most strongly adsorbed (*2*, *15*). The moment of *p*-benzoquinone monoxime in dioxane solution has been reported to be 4.72 *D* (*7*). This compound is adsorbed between the *o*- and *p*-dioximes. Data on the electric moment of the *o*-monoxime are not available. However, a direct correlation between dipole moment and adsorbability should not necessarily be expected in this case, owing to possible shifts in the tautomeric equilibria. The *o*-monoxime is adsorbed as a red band at the top of the column, while the para isomer appears as a green band. This suggests that the ortho compound is adsorbed in the quinoid form, while the para isomer is adsorbed as *p*-nitrosophenol, for the green color is similar to that of compounds containing the free nitroso group (*14*). The proximity of the functional groups in the ortho compound, on the other hand, might lead to chelation with the adsorbent. This would tend to shift the equilibrium to the quinoid modification.

A more direct correlation is observed between the order of adsorption and the wave lengths of the spectrophotometric maxima in alkaline solution. In this case the compounds which absorb light of longer wave length are bound more tightly. It is evident that the compounds are polarized by contact with the alumina, for the free acids are pale yellow to almost colorless. While the formation of colored bands is not requisite to a good separation, it is of great practical convenience in locating and distinguishing between the adsorbed compounds.

Owing to restricted rotation about the C=N bond, the existence of cis-trans isomers of the benzoquinone dioximes was considered possible. However, under the experimental conditions described, the bands appear homogeneous and no differences in properties can be detected between materials eluted from the upper and lower portions of the columns.

The choice of the solvent, adsorbent, and eluant was guided by a consideration of the physical properties of the compounds. Acetone is the only solvent readily available in which they are easily soluble and from which they can be adsorbed by alumina. However, it is difficult to free from traces of water and alcohol and undergoes a condensation reaction in contact with the adsorbent which results in the presence of a high-boiling liquid in the percolate (*18*). These factors may cause variations in the widths of the bands, and complicate recovery of the impurities found in the percolate, but otherwise do not interfere with the analysis. Alumina, magnesia, talc, silica gel, and a number of other materials were tested as adsorbents, but, aside from alumina, magnesia was found to be the only one of potential value. Of the possible eluants, aqueous sodium hydroxide is the most effective. The compounds are readily removed from the adsorbent and optical measurements can be made directly on the filtered and diluted solutions without further adjustments.

The chromatographic adsorption method of Tswett (*16*) has found extensive use in the purification and estimation of naturally occurring substances. However, applications in the field of industrial organic analysis have been comparatively few. This may in part be ascribed to the relatively poor precision obtained when compared to standard gravimetric and volumetric methods and to the small quantities of materials which can be isolated on columns of convenient size. However, when used in conjunction with absorption spectrophotometry, the usefulness of both methods is greatly extended, for the small amounts of material recovered from the column can often be measured conveniently while frequently compounds can be separated which would otherwise produce unresolved absorption spectra. While optical methods have been proposed in which a number of constituents are determined from a series of measurements made on a complex reaction mixture, it is evident that they are completely valid only in cases where the qualitative composition of the mixture can be predicted with certainty. If the properties of the materials permit, a preliminary separation will frequently augment the reliability of the analysis.

EXPERIMENTAL

p-Benzoquinone monoxime was prepared by the nitrosation of phenol, and purified by repeated recrystallizations from water until a pale yellow product was obtained with a melting point of 126° C.

o-Benzoquinone monoxime was prepared by the oxidation of phenol with hydrogen peroxide in the presence of cupric acetate and hydroxylamine hydrochloride (*4*). The reaction mixture was acidified and extracted with petroleum ether, and the monoxime concentration determined iodometrically (*6*). Spectrophotometric measurements were made on a solution obtained by extracting an aliquot of the petroleum ether with aqueous sodium hydroxide.

p-Benzoquinone dioxime was prepared by oximation of the monoxime with hydroxylamine acid sulfate. The crude product was adsorbed on alumina, the zones containing impurities removed and discarded, and the dioxime displaced by treatment with a solution of acetic acid in acetone. After evaporation of the solvent, the product was further purified by precipitation from acetone solution by the addition of petroleum ether. A pale yellow product was obtained with a decomposition point of 239° C.

o-Benzoquinone dioxime was prepared by the oxidation of *o*-nitraniline with sodium hypochlorite (*9*), followed by reduction of the benzfurazan oxide with sodium hydrosulfide. The crude product was dissolved in aqueous ammonia, filtered, and precipitated by the addition of acetic acid. Yellow needles with a melting point of 145° C. were obtained.

ACKNOWLEDGMENTS

The methods for the gravimetric determination and purification of *p*-benzoquinone dioxime were devised by H. P. C. Burrell. Many of the spectrophotometric measurements were made by Virginia S. Martin.

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Determination of Free Fatty Acids in Dried Egg Powders

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A method for the determination of free fatty acids in dried egg powders is described. The egg powders are extracted with acetone and the traces of cephalin are removed with magnesium chloride before titration. The removal of the cephalin, previously shown to be responsible for as much as 70% of the total acidity of ether extracts of egg powders, facilitates accurate measurement of small amounts of free fatty acid acidity. The acidity of extracts of egg powders from which cephalin has been removed by the acetone–magnesium chloride treatment is not appreciably influenced by variations in the moisture content of the powder (at least within the range 0.3 to 6.5%). Errors caused by the formation of a fatty acid–protein complex have been studied. Fatty acids are bound by basic proteins in a nonextractable form when the egg is in the liquid state or when dried egg powder is reconstituted. The fatty acids thus bound can be recovered completely only if the egg emulsion is adjusted to pH 4 before drying and extracting. Fatty acids which develop in the dry egg powder during storage are not bound and can be completely extracted by the proposed method.

A RECENT paper (*9*) was concerned with the differences between the total acidity of the ether extract of dried egg powders as determined by the A.O.A.C. method (*4*) and the free fatty acid acidity of the powders. In some cases over 70% of the acidity of the ether extract of unstored egg powders was found to be due to cephalin. The amount of cephalin appearing in the ether extract, in contrast to the free fatty acid content, was influenced by the moisture content of the powder, so that it was necessary that the powders be uniformly dried, as specified in the A.O.A.C. procedure, in order to obtain reliable total acidity values.

When recovery experiments were carried out (*9*), it was found that oleic acid, that had been added to liquid egg mixtures, was apparently bound in some way that prevented its extraction from the corresponding dried egg powders unless the liquid egg was adjusted to about pH 4 before drying. Thus it appeared that reliable free fatty acid values for the dried egg powders could be obtained only by reconstituting the dried egg, acidifying to pH 4, and redrying prior to extraction. In addition, a correction for the cephalin content of the extract was necessary.

This paper describes a more satisfactory method of determining the true free fatty acid content of dried egg powders and presents additional information on the combination of free fatty acids in the liquid whole egg.

The proposed method makes use of the differential solvent power of cold (room temperature) acetone. Glycerides and fatty acids are fully soluble in cold acetone, whereas the phospholipides (phosphatides) are only slightly soluble. Traces of dissolved phospholipide are removed by treatment with alcoholic magnesium chloride (*8*). The phospholipide concentration is thus reduced to negligible amounts, the phosphorus content of the treated oil is usually less than 0.01%.

ANALYTICAL PROCEDURE

A 2-gram sample of the dried egg powder was transferred to a 50-ml. conical, graduated centrifuge tube, and 15 ml. of C.P. acetone were added. The sample was centrifuged after it had stood in the acetone for 0.5 hour. The acetone extraction was repeated four times, using 10-ml. portions of acetone and only 5- to 10-minute extraction times. The acetone suspension of the egg powder was frequently stirred during each of the extraction periods. All the extracts from a given sample were combined in a single centrifuge bottle. The final volume of acetone extract was kept below 60 ml.

One milliliter of a saturated solution of magnesium chloride hexahydrate in absolute ethanol was then added with rapid swirling to the combined extracts. The treated acetone solution was centrifuged and the supernatant liquid transferred to a 500-ml. separatory funnel (with stem cut to 0.5 inch, 1.25 cm.). The magnesium chloride–phospholipide precipitate was washed twice by suspending in 5 ml. of acetone and centrifuging. The washings were combined with the original supernatant in the separatory funnel.

Thirty milliliters of petroleum ether (Skellysolve B) were added to the acetone solution in the separatory funnel and thoroughly mixed. Two hundred to 250 ml. of distilled water were then added and the contents of the funnel were agitated gently to prevent emulsion formation. The volume of acetone in the separatory funnel should be maintained as small as possible (60 to 80 ml.) because the acetone concentration in the washing procedure must lie between 20 and 30% in order to prevent losses of fatty acids in the washing step. This means that 200 to 250 ml. of water must be added if the initial acetone volume is 70 to 80 ml.

After separation of the phases, the aqueous acetone was discarded. The aqueous phase was turbid, owing to traces of magnesium chloride and phospholipide. The petroleum ether was washed with two 25-ml. portions of distilled water. The washings gave a negative chloride test. The petroleum ether solution was then transferred quantitatively to a 125-ml. glass-stoppered Erlenmeyer flask.

Fifty milliliters of isopropanol and 10 drops of 1% ethanolic phenophthalein were added, and a stream of carbon dioxide–free air was bubbled through the solution for 10 minutes. The solution was titrated with 0.025 *N* sodium ethylate with the stream of carbon dioxide–free air passing through the solution. Oleic acid has been reported to constitute approximately 50% of the egg fatty acids (*10*). Therefore, as a matter of convenience, the results were calculated as oleic acid.

RESULTS AND DISCUSSION

RECOVERY OF OLEIC ACID ADDED TO DRY EGG POWDERS. Recovery of oleic acid was determined by adding known amounts of oleic acid in acetone solution to samples of a spray-dried egg powder suspended in acetone. These samples and controls were carried through the procedure as outlined above. The data in Table I show satisfactory recovery of the added acid.

ANALYSIS OF STORED EGG POWDERS. Samples of spray-dried egg powders, containing only the free fatty acids developed during storage in the dry state, were analyzed by the proposed method. Samples of the same egg powders were also exhaustively extracted with diethyl ether in a Soxhlet apparatus, the ether removed from the Soxhlet extracts under a stream of nitrogen, and the residual oil taken up in acetone and subjected to the magnesium chloride treatment and washing as outlined above. Thus only the effect of different types of extraction was studied. The results in Table II indicate that the shorter acetone extraction procedure yields results from 2 to 7.5% lower, but an error of this magnitude is probably permissible for many purposes. In some cases, the more time-consuming ether Soxhlet extraction may be desirable.

The phospholipide content of all extracts treated with magnesium chloride was negligible, as evidenced by phosphorus contents

Table I. Recovery of Oleic Acid Added to Spray-Dried Egg Powder

Acid Initially Present as Oleic Acid	Oleic Acid Added[a]	Acid Recovered as Oleic Acid	Recovery
Mg.	*Mg.*	*Mg.*	%
10.0, 9.8, 10.1, 10.1	5.0	15.1, 15.1, 15.1, 15.1	101.5
10.0, 9.8, 10.1, 10.1	25.1	34.0, 34.4, 34.3, 34.6	96.4
10.0, 9.8, 10.1, 10.1	50.2	59.0, 59.4, 57.6, 58.5	96.6

[a] Added in acetone solution to acetone suspension of egg powder sample in first step of procedure.

Table II. Free Fatty Acid Values of Dried Egg Powders as Influenced by Extraction Method and Pretreatment

Sample	Treatment	Oleic Acid	Average	Standard Deviation
		Mg. per gram of dry powder		
A	Soxhlet extraction with ether	20.7, 21.2, 21.0 20.8, 20.2, 20.5, 20.8	20.8	0.3
A	Proposed method	20.6, 19.6, 19.9 18.5, 18.6, 19.0, 18.6	19.3	0.8
A	Sample adjusted to pH 4.0 Soxhlet extraction with ether	19.4, 19.6, 19.5, 19.8 19.7, 19.9, 19.6, 20.0	19.7	0.2
A	Sample adjusted to pH 4.0 Proposed method	18.5, 19.1, 19.4, 19.4 19.3, 19.0, 19.2, 18.8	19.1	0.3
C	Soxhlet extraction with ether	3.82, 3.94	3.88	....
C	Proposed method	3.50, 3.52, 3.73, 3.63, 3.52, 3.65 3.49, 3.55, 3.55, 3.54, 3.72, 3.65	3.59	0.08
C	Sample adjusted to pH 4.0 Soxhlet extraction with ether	3.47, 3.57, 3.52, 3.54	3.52	0.03
C	Sample adjusted to pH 4.0 Proposed method	3.37, 3.47, 3.47	3.44	....

(method of Allen, *3*) of 0.001 to 0.01%. Cephalin, therefore, could make only a very small contribution to the acidity values of these extracts.

Effect of Moisture Content of Powders on Free Fatty Acid Values. The proposed method does not require preliminary drying of the egg sample prior to extraction. Average free fatty acid values, calculated to the moisture-free basis, were 17.1, 16.8, 16.9, and 16.6 mg. of oleic acid per gram of powder at moisture levels of 6.5, 4.9, 1.8, and 0.3%, respectively. This relative constancy of results over the moisture range was expected, since the cephalin content of the extracts was negligible as the result of the magnesium chloride treatment. However, the total acidity of ether extracts that are not treated with acetone and magnesium chloride may vary as much as 30% over comparable moisture ranges. This variation has been shown (*9*) to be due to the different amounts of cephalin extracted by ether at the different moisture levels. This, of course, is the reason for the preliminary drying of the samples in the A.O.A.C. procedure for the acidity of the ether extract.

Errors Resulting from Fatty Acid–Protein Complex Formation. Another possible source of error in the free fatty acid determination in egg powders was suggested by the observation that oleic acid, which had been added to whole liquid egg mixtures, was incompletely recovered in the ether extract of the subsequently dried powder unless the pH of the egg mixture was adjusted to 4 prior to drying (*9*).

A similar situation exists for acetone extraction. Only 50 to 55% of the oleic acid added to liquid egg mixtures was extracted by neutral acetone. Therefore, a modified procedure based on the use of acidified acetone (0.088 *N* with respect to hydrochloric acid) was tried on samples to which oleic acid was added before drying. The acidified acetone was used in the first two extractions and neutral acetone in the last three extractions. Acidified acetone was not used in the last three extractions, since high results due to the extraction of phosphorus compounds that were not removed by the magnesium chloride treatment were obtained. Acidified acetone in the first two extractions did not extract phosphorus compounds that could not be removed by magnesium chloride.

Although the pH of the suspension of the residues from the acid acetone extraction was near 4.0, only 80 to 90% recovery could be attained by this technique. Therefore, the acid acetone extraction is not recommended as a means of obtaining complete extraction of fatty acids from egg samples which may contain bound fatty acids. Rather, the procedure involving reconstitution, adjusting to pH 4, and lyophilizing is recommended in these cases. The fatty acids in the dried, adjusted powder may then be determined by the proposed method.

Although the recovery of oleic acid added to or present in liquid egg mixtures prior to drying is not complete unless the pH of the liquid mixture is adjusted to pH 4.0, extraction is complete from egg powders which have develope free fatty acid acidity while stored in t dry state. Tables II and III show that t recovery of fatty acids, by the propos method, is not increased by the adjustme of the acidity of samples of either spra dried or lyophilized egg powders to pH

If, on the other hand, samples of e powders, in which acidity had develope are reconstituted and allowed to stand the liquid state at its natural pH for time before being lyophilized, then pa of the fatty acids are no longer extrac able. For example, a suspension of reco stituted egg powder had a pH of 6.7; aft redrying by lyophilizing, only 86% of t fatty acids were recovered. Recovery free fatty acids from a similar samp which had been adjusted to pH 4 was complete.

These results may be explained by assuming that liberation free fatty acids in the dry egg powder occurs in a substantial fatty medium—i.e., the fatty acids are, in a microscopic sens in or near the egg lipides—and, for this reason or because the m dium is essentially nonaqueous, the free fatty acids have little no opportunity to combine with bases present in the egg. Wh fatty acids are present in the liquid egg mixture, they are free combine with bases to an extent determined by the pH of t mixture.

It might be expected that egg white would bind fatty acids mo strongly than the egg yolk, since the proteins of the white conta many basic groups that may combine with fatty acids. Lys zyme, in particular, is an unusually basic protein constituent egg white (*2*) which has been shown to form salts with stro acids (*1*). The higher fatty acids would doubtless also form sal with lysozyme and would hardly be expected to be removed fro the lysozyme in the pH region of 6 to 8 which is midway betwe the isoelectric point of lysozyme and the pK of the acids. Su port for this assumption is also obtained from reports (*5*, *6*) th the lower fatty acids and serum albumin combine in solution give complexes that are more heat-stable than serum albumin di solved in solutions containing only inorganic ions. These worke have also presented evidence, based on electrophoretic measur ments, for anion-albumin association which increases with cha length of the anion.

An experiment was performed to determine whether there was difference in the fatty acid binding capacity of egg white and eg yolk. Known amounts of oleic acid were added to fresh mixe yolks and the fresh mixed whites. Portions of each sample we adjusted to pH 8 and also to pH 4 before drying by lyophilizatio At pH 8, only 4% of the added oleic acid was recovered from th whites, while 81% was recovered from the yolk. At pH 4, 84 of the oleic acid was recovered from the whites and 96% from th yolks.

The formation of the nonextractable fatty acid–protein com plexes is of analytical significance in the case of samples of u known history or in those cases where opportunity for contac

Table III. Free Fatty Acid Content of Stored Lyophilized Eggs a Function of pH[a]

Sample	pH	Oleic Acid *Mg./g.*	
1	7.7	10.9	10.6
	4.0	11.0	10.4
2	7.5	11.5	11.4
	3.8	11.2	11.4
3	7.4	10.0	10.0
	3.7	10.0	9.9
4	7.1	11.4	11.1
	4.1	11.2	11.2

[a] pH of suspension of 1 part egg powder and 3 parts distilled water.

between fatty acids and proteins in aqueous media has been known to exist. Thus the free fatty acids in shell eggs which have undergone some deterioration would have an opportunity to combine with protein and the pH should be adjusted to 4 prior to drying the sample.

The lowest moisture content at which interaction of the fatty acids and proteins occurs is not known. However, egg powders containing as much as 8% moisture gave the same free fatty acid values before and after adjustment of the pH. Since few dried egg samples are prepared at higher moisture levels, no adjustment of the pH of dried powders that have been prepared from good shell eggs seems necessary.

INTERFERENCES AND LIMITATIONS

The production of lactic acid in certain types of egg deterioration has been reported (*7*). Lactic acid is, of course, easily soluble in water and is not determined in the method outlined above. Amounts of lactic acid corresponding to 14 to 50 mg. per gram of egg powder were added to samples of the powder and were shown to make no contribution to the acidity of the samples.

The method would not be applicable to samples of materials likely to contain fatty acids that are water-soluble, since they would probably be incompletely partitioned between the aqueous acetone and the petroleum ether in the washing process, thus leading to low results. Since the lower fatty acids have been shown to be absent from egg fat (*10*), such losses do not occur in the determination of egg fatty acids arising from the usual type of glyceride hydrolysis.

Moderate excesses of acids, added to lower the pH of the sample, do not interfere, since they are removed during the washing procedure.

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Iodometric Method for the Assay of Penicillin Preparations

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TWO methods for the chemical estimation of penicillin, recently proposed (*4*, *5*), depend on the liberation of an acidic group upon inactivation of penicillin with penicillinase or with alkali. The simple, rapid inactivation by alkali was being utilized in this laboratory in conjunction with the development of an iodometric assay method which was suggested by observations (*3*) on the behavior towards iodine of penicillin and certain of its inactivation products. Penicillin is inert to iodine in neutral aqueous solution, while penicilloic acid (*1*), the inactivation product formed from penicillin by the action of penicillinase or of alkali, consumes from 6 to 9 equivalents of iodine, depending on the conditions used. The difference in the iodine consumption under standard conditions before and after inactivation by alkali might therefore be expected to be approximately proportional to its penicillin content as ascertained by biological assay. This was found to be the case, with certain limitations.

EXPERIMENTAL

Iodine Equivalent of Pure Sodium Penicillin G Inactivated with Alkali. The iodine consumption of a neutral solution of alkali-inactivated sodium penicillin G is a function of time, and under the conditions given below becomes constant after 25 to 30 minutes.

A weighed sample (3 to 5 mg.) of crystalline sodium penicillin is place in a glass-stoppered flask, dissolved in 5 to 10 cc. of water, and treated with 0.5 cc. of 1 *N* sodium hydroxide. The alkaline solution is allowed to stand for 15 minutes and then neutralized with 0.5 cc. of 1 *N* hydrochloric acid. A measured excess of 0.01 *N* iodine solution (about 10 cc.) is added. After minutes the unconsumed iodine is titrated with 0.01 *N* thiosulfate. It was found that 1 mg. of sodium penicillin G when activated will consume under these conditions 2.52 cc. of 0.01 *N* iodine solution, corresponding to 8.97 equivalents of iodine per mole, which is in reasonably good agreement with the range given for penicilloates (8.5 to 8.9). This value is well reproducible and independent within fairly wide limits of the penicillin concentrations and the excess iodine used.

Sodium penicillin F (crystalline, anhydrous) after inactivation treated in this manner consumed 2.64 cc. of 0.01 *N* iodine solution per milligram, or 8.8 equivalents per mole, showing that the double bond in this compound is inert to iodine under these conditions. This was confirmed by the blank titration (without prior alkali treatment) which showed an uptake of only 0.07 cc. per milligram. However, it is probable that penicillin X might give abnormally high values in this procedure.

Assay of Unknowns. In the assay of unknowns an amount of material corresponding to approximately 1000 to 5000 units is inactivated with alkali, and the neutralized solution is treated with iodine and back-titrated after 30 minutes as described above for pure sodium penicillin G. A blank titration in which the treatment with alkali is omitted is carried out on a separate sample of the same magnitude. The difference between both titrations can be used, as indicated below, to calculate either international units or micromoles of penicillin.

When relatively pure preparations (800 to 1000 micrograms per mg.) were assayed in this manner, the figures agreed well with the bioassay values regardless of the time allowed for contact of the blank sample with excess iodine (30 minutes as in inactivated sample, or immediate back-titration), the small amounts of iodine-consuming impurities present in such preparations having little influence on the final result. However, with preparations of lower potency containing proportionately greater amounts of iodine-consuming impurities, the iodometric results were considerably lower than the bioassay figures when the 30-minute interval between addition of iodine and back-titration was maintained also in the blank determination. A possible explanation for this is that products formed from these impurities by reaction with iodine catalyze the slow hydrolysis of penicillin to penicilloic acid normally occurring even in neutral reaction, giving rise to an erroneously high blank value, since the latter would include the penicilloic acid thus formed from penicillin. This assumption is supported by the finding that the addition of crystalline penicillin to an impure sample increases the blank titration (after 30 minutes' contact with excess iodine) over the blank value (also determined after 30 minutes standing) given by the impure sample alone.

It was found empirically that the discrepancy between iodometric and bioassay values in the case of low-grade preparations was minimized when the blank value arrived at by immediate back-titration was used in computing the results. In the final

Table I. Correlation between Iodometric and Bioassay Values

Sample No.	Iodometric Assay γ/mg.	Cup Test γ/mg.
890	790	800
	816	
	800	
1080	942	968
	964	
	947	
A69-71	120	100
	107	
	126	
D041	495	502
	492	
	468	
D042F-1	345	336
	330	
	340	
1088 u.u.f.	None	None
DE43	530	480
	507	

Table II. Comparison of Biological and Chemical Assay Methods on Solutions of Commercial Penicillin Sodium Salts

	Biological		Chemical	
Na Salt Solution	2-cc. test (2)	Cup test	Iodometric	Alkalimetric[a]
	Micrograms per cc.			
3419	90,900	71,400	88,000	90,000
3422	87,100	85,000	92,500	91,600
3423	90,400	81,000	88,800	81,000
3420	107,300	90,300	112,500	90,400
3424	87,000	70,700	88,500	...
3425	89,000	77,600	91,500	89,300
3426	70,000	68,700	83,600	66,300
3427	86,000	79,000	84,000	92,000
3505	101,603	94,500	98,800	99,200
3506	113,700	108,700	105,500	106,000
3428	96,300	89,100	100,200	81,200
3429	96,500	89,300	101,000	90,700
3430	97,900	88,500	94,500	94,500
3431	85,000	80,300	88,000	87,000
3507	110,000	106,800	114,400	111,500
3428-2	90,900	73,700	83,600	92,300
3432	72,089	74,000	76,500	86,100
3433	93,200	92,900	98,500	107,500
3434	96,400	93,000	98,000	107,600
3435	103,600	105,200	99,300	95,200
3436	116,900	114,000	112,500	114,200
3437	70,900	69,400	68,000	68,800

[a] Method to be published.

procedure adopted for the assay of high- as well as low-grade preparations, the blank sample is therefore back-titrated immediately after the addition of the iodine solution, whereas with the alkali-inactivated sample the 30-minute interval is maintained.

Example. A sample of 6.386 mg. of a commercial penicillin salt (bioassay 800 micrograms per mg.) was dissolved in water and the volume was made up to 10.0 cc. A 5-cc. aliquot was pipetted into a 125-cc. glass-stoppered flask, and 10.0 cc. of a 0.01 *N* iodine solution were added. Immediate back-titration with 10.0 *N* thiosulfate showed that 0.92 cc. of 0.01 *N* iodine had been consumed. When the back-titration was delayed for 30 minutes, practically the same result (0.95 cc.) was obtained.

The remainder of the solution (together with the pipet washings) was made alkaline with 0.50 cc. of 1 *N* sodium hydroxide. After standing for 15 minutes, the solution was neutralized with 0.50 cc. of 1 *N* hydrochloric acid, treated with 10 cc. of 0.01 *N* iodine solution, and back-titrated after 30 minutes. The iodine uptake corresponded to 4.86 cc.

Calculation. Potency (units per mg.) =

$$\frac{(4.86 - 0.92) \times 661}{3.193} = 816$$

The factor 661 represents the ratio of the potency of sodium penicillin G (1667) to the number of cubic centimeters of 0.01 *N* iodine consumed by 1 mg. of alkali-treated sodium penicillin G (2.52).

With the use of this procedure good correlation between the iodometric and bioassay values was obtained on a variety of carefully bioassayed solid sodium salt preparations of widely varying potency (Table I). Table II gives a comparison of the values obtained by the iodometric, the alkalimetric (*5*), and two standard bioassay methods This survey was conducted in the Squibb Biological laboratories on solutions of commercial sodium salt preparations ranging in potency from 900 to 1150 units per mg. The figures show that the agreement between the two chemical methods, and between the latter and the 2-cc. test is in most cases satisfactory enough for practical purposes. It is possible that the iodometric method can be used for rapid control work in the intermediate stages of the plant process, but no data on such materials are as yet available.

The iodometric procedure has certain advantageous features not possessed by the alkalimetric method. It requires less material than the latter, and therefore can be applied to more dilute solutions; it also can be adapted to the assay of buffered solutions, which is valuable particularly in chromatographic work.

Finally, the comparison of bioassay figures with the iodometric values, or for that matter with the results obtained by any assay method which measures the number of penicillin molecules, is predicated on the condition that the penicillin in the unknown sample be of the same type as in the pure standard preparation used in both kinds of assay. Since the various penicillin species (F, G, X, K, etc.) differ somewhat in molecular size, the iodine consumed after alkaline inactivation will be inversely proportional to the molecular weight. Consequently, when the composition of the unknown sample deviates materially from that of the standard in regard to the type or types of penicillin present the iodometric value for the unknown will not correspond exactly to its penicillin content if the latter is expressed in terms of milligrams. However, the errors caused by this circumstance are comparatively small and may be disregarded in practice.

A more serious complication is encountered when the iodometrically measured penicillin content of an unknown sample is expressed in terms of international penicillin units and then compared with the unitage ascertained by biological assay. It is well known that the various penicillin species differ widely in their bacteriostatic potency towards the test organism commonly used, *Staph. aureus*. Thus the potencies of the sodium salts of penicillins F, G, and K determined by standard assay methods employing this test organism are 1400, 1667, and 2350 units per mg., respectively. It is obvious that when the unitage of an unknown preparation is computed from the iodometrically determined penicillin content by means of the conversion factor 1 mg. = 1667 units for sodium penicillin G (the International Standard), marked discrepancies from the values ascertained by bioassay are to be expected if penicillins other than G are present as major components. Since quantitative information on this latter point is hardly ever available, the applicability of this as of any chemical method is necessarily restricted to the comparative assay of products similar in respect to derivation and mode of preparation. Therefore, in cases in which the measurements reveal a consistent discrepancy between the chemical and the bioassay data, it is preferable to base the computation of unitage from the chemical assay on a conversion factor empirically determined with an impure standard preparation of the same type rather than on the factor for pure sodium penicillin G given above.

ACKNOWLEDGMENT

The author is indebted to H. A. Holaday, general superintendent and director of the Squibb Biological Laboratories, for making available the data in Table II, and to Florence T. Starkey who carried out the iodometric and cup assays listed there. Appreciative acknowledgment is made to Oskar P. Wintersteiner for his advice and interest in this work.

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Spectrophotometric Changes during Oxidation of Vitamin A Oils

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Both the quality of the carrier oil and the quantity of vitamin A are important for the evaluation of fish liver oils containing vitamin A. The former develops maxima at 235 and 280 mμ during oxidation. These can be used advantageously for estimating the quality of fish liver oils by measuring the ratio $E^{1\%}_{1cm.}$ 280/328, and from the percentage by which the absorption of the unsaponifiable fraction is lower than that of the whole oil at 280 mμ. The quantity of the vitamin A in oils of high potency can be estimated from the unsaponifiable fraction absorption at 325 mμ since the unsaponifiable fraction does not change appreciably during the early stages of oxidation.

FISH liver oils containing vitamin A are essentially solutions of the esters of that vitamin in fatty oil (carrier oil). It may be divided into two fractions, the saponifiable and the unsaponifiable carriers—i.e., the portions respectively nonextractable and extractable with ether after saponification of the oil. Knowledge of the quality of the carrier oil is of utmost importance for the over-all evaluation of vitamin A oils, since carrier oils containing degradation products due to oxidation have considerable influence on the extinction coefficient at 328 mμ and the whole vitamin A curve. While the vitamin A ester itself has a sharp maximum at 328 mμ, biologically inactive substances, which may accompany it, do not show this sharp peak, but have maxima at wave lengths sufficiently close to 328 mμ to produce considerable absorption at this point, thus causing an apparent broadening of the vitamin A absorption band and an erroneously high value at this point. Oxidized oils can also interfere with the vitamin A absorption in vivo (*7*, *12*, *13*, *16*) and so inhibit its action.

The method of Oser *et al.* (*18*) of plotting absorption curves of vitamin A oils in terms of absorption ratios ($E^{1\%}_{1\ cm.}$ λ328) could be useful in detecting anomalous or oxidized oils. However, the method for measuring the ratio $E^{1\%}_{1\ cm.}$ 300/328 with the limit of 1.72 for acceptable oils (*11*, *19*) did not prove satisfactory. Work in this laboratory had disclosed the fact that an oil could be considerably oxidized and still show a satisfactory $E^{1\%}_{1\ cm.}$ 300/328 ratio and Oser *et al.* (*19*) showed that oils with an acceptable $E^{1\%}_{1\ cm.}$ 300/328 ratio did not always show the biological potency corresponding to their $E^{1\%}_{1\ cm.}$ value at 328 mμ.

It therefore seemed desirable to study spectrophotometric curves of both components of the carrier oil, apart from vitamin A, during accelerated oxidation, in an effort to establish their influence upon the absorption curve of vitamin A oils. Such a study could also be helpful in developing better criteria for the evaluation of vitamin A oils.

EXPERIMENTAL

Outline of Procedure. Freshly prepared oils were oxidized at 75° C., and the absorption curves measured in the region from 220 to 400 mμ on both the whole oil and the unsaponifiable fraction, the difference between the two representing the absorption of the saponifiable carrier. The absorption of a part of oxidized vitamin A which is washed out during the saponification procedure (*18*) is also included in the subtracted curve. In order to obtain the curve of the unsaponifiable carrier, the unsaponifiable fraction was destructively irradiated. On the assumption that only vitamin A is being destroyed by irradiation, the absorption curve obtained after irradiation would represent the absorption of the unsaponifiable carrier. By subtracting the absorption values of the unsaponifiable carrier from those of the unsaponifiable fraction a vitamin A curve can be obtained representing essentially the pure vitamin A absorption. The values so obtained are referred to as the modified values (*17*).

Preparation of Oils. Two test oils were used: a grayfish (*Squalus suckleyi*) liver oil and a ling cod (*Ophiodon elongatus*) liver oil. The grayfish liver oil was prepared by steaming minced fresh livers with live steam for 10 minutes, and then centrifuging; the ling cod liver oil by similarly steaming minced fresh livers for 10 minutes, extracting the steamed livers with a mixture of methylene chloride and ethylene dichloride, and distilling off the solvent in vacuum. The oils showed a very high stability. The grayfish oil especially had a stability far in excess of that observed on commercially prepared grayfish liver oils.

Apparatus, Reagents, and Procedures. The oxidation of the oils was carried out at 75° C. in a constant-temperature oil bath, using the following technique: From 0.33 to 0.36 gram of the oil was introduced into vials of 23-mm. outside diameter and 25 mm. high. These were then placed in a frame bored with holes which, when lined with felt, were of a suitable size to hold the vials firmly upright. The frame was kept immersed in an oil bath maintained at a constant temperature of 75° C. For each oxidation 4 to 10 vials were used. The oils were heated for varying lengths of time in order to obtain oils at different stages of oxidation. The oxidized oils from these vials were afterwards combined and kept for analyses in full ampoules at −20° C.

The spectrophotometric analyses were carried out in duplicate with a Beckman quartz spectrophotometer, using an ultraviolet phototube and, as light source, a hydrogen discharge tube below 320 mμ and a tungsten lamp above 320 mμ. All determinations were made in quartz cuvettes with isopropanol as solvent. The latter was purified by refluxing for one hour with zinc dust and sodium hydroxide, and then distilling.

The saponifications were carried out essentially according to the method of Oser *et al.* (*18*). Blank saponifications made on the reagents were used as blanks for the spectrophotometric determinations. The irradiation of the saponified samples was carried out with a Uviarc lamp UA 32A6, and a Corning filter No. 597. The quartz cuvette containing the material to be irradiated was placed in a water-cooled holder at a distance of about 20 cm. from the lamp and irradiated for 1 hour. Corning filter 597 does not give such a sharp maximum as the arrangement used by Little (*17*) but has a flat maximum at about 350 mμ and cuts out all wave lengths below 300 mμ.

DISCUSSION OF RESULTS

In Figures 1 to 10 are plotted the absorption curves of both test oils; the numbers on the individual curves refer to hours heated at 75° C. In Table I are collected those characteristic values which were considered important for the present investigation.

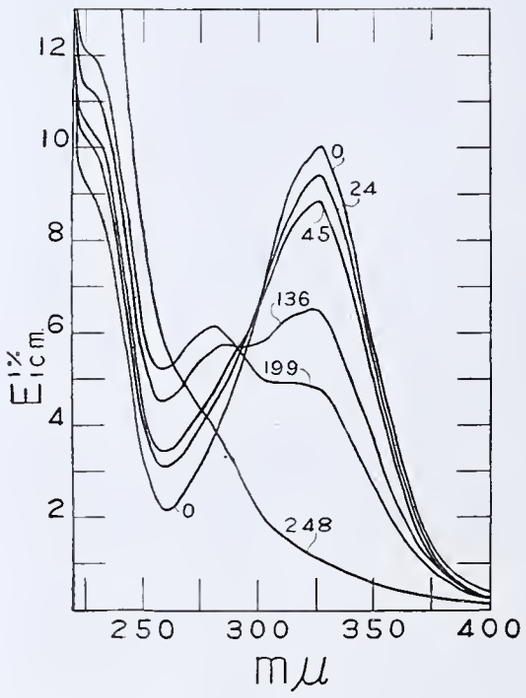
Saponifiable Carrier. In Figures 4 and 9, representing the absorption of the saponifiable carrier, it can be seen that during oxidation new maxima at 235, 275 to 280, and 335 to 345 mμ are formed. The latter increases at first but decreases in the later stages of oxidation, indicating instability of the substance which causes this maximum. Formation of the maximum at 335 to 345 mμ could be due to oxidation of highly unsaturated acids which are known to be present in fish liver oils.

The shape of the curves of the oxidized saponifiable carrier of both test oils resembles the curves of rancid and oxidized fats. Holman *et al.* (*14*, *15*) found the formation of the 235 and 275 to 280 mμ maxima during oxidation of dienoic and trienoic acids and of lard. Bradley (*2*) made a similar observation on heat-bodied linseed oil, Edisbury *et al.* (*8*) on ling oil, and Castle *et al.* (*4*) found that vitamin A carefully oxidized with chromic acid absorbs at 275 to 280 mμ.

Table I. Characteristics of Test Oils durin

Time of heating, hours	Whole Oil: Vitamin A estimate[c]	Whole Oil: Decrease in vitamin A estimate, %	Whole Oil: Maximum at mμ	Whole Oil: F.F.A., %	Whole Oil: Peroxide value	Whole Oil: $E^{1\%}_{1\ cm.}$ 260/328	280/328	300/328	350/328	Unsaponifiable: Vitamin A estimate[c]	Unsaponifiable: Decrease in vitamin A estimate, %	Unsaponifiable: Maximum at mμ	Unsaponifiable: $E^{1\%}_{1\ cm.}$ 280/328	300/328	350/328
Grayfish Liver oil (Unsaponifiable 21.0%)															
Original	19,930	0	327	0.46	0.2	0.22	0.37	0.67	0.57	19,620	0	325	0.34	0.68	0.50
24	18,600	6.7	326	0.64	1.9	0.33	0.47	0.71	0.58	17,660	10	325	0.36	0.68	0.51
45	17,490	12.2	325	0.71	3.1	0.39	0.54	0.74	0.57	16,200	17.4	325	0.38	0.70	0.51
136	12,780	35.9	323	0.91	5.1	0.71	0.87	0.90	0.58	10,380	47.1	325	0.40	0.71	0.53
199	9,300	53.3	281	1.21	5.6	1.13	1.32	1.09	0.57	6,900	64.8	325	0.58	0.81	0.52
248	2,080	89.6	...	5.79	62.4	5.53	3.88	2.12	0.56	316	98.4	280	3.68	2.84	0.60
Ling Cod Liver Oil (Unsaponifiable 10.5%)															
Original	68,820	0	328	2.3	0.9	0.14	0.28	0.58	0.62	65,040	0	325	0.26	0.59	0.54
6	67,740	1.6	328	2.6	2.3	0.16	0.30	0.59	0.62	61,640	5.2	325	0.28	0.61	0.54
18	64,340	6.5	328	2.6	4.8	0.22	0.36	0.63	0.61	55,420	14.8	325	0.29	0.61	0.54
24	55,160	19.8	328	2.6	5.8	0.29	0.43	0.67	0.62	44,560	31.5	325	0.33	0.63	0.55
32	33,440	51.4	327	2.8	11.7	0.63	0.78	0.84	0.58	26,800	58.7	325	0.40	0.70	0.54
40	20,800	69.8	280	2.9	16.4	1.19	1.35	1.20	0.59	15,580	76.0	325	0.56	0.77	0.53
72	8,960	87.0	275	3.7	33.0	3.56	3.50	2.20	0.61	1,540	97.6	...	2.70	1.88	0.55

[a] Unsaponifiable minus irradiated unsaponifiable.
[b] Per cent of absorption of whole oil.
[c] Determined spectrophotometrically.



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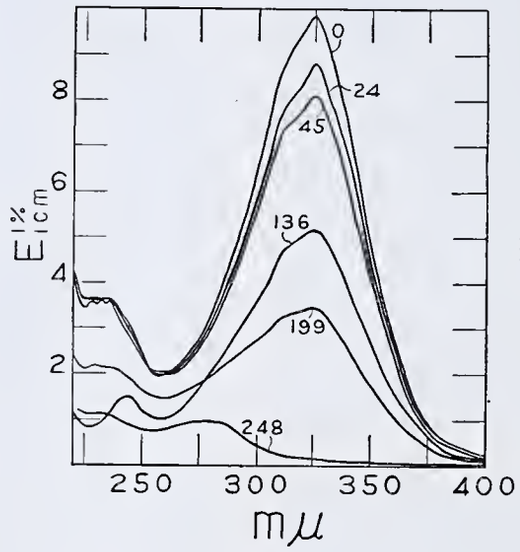

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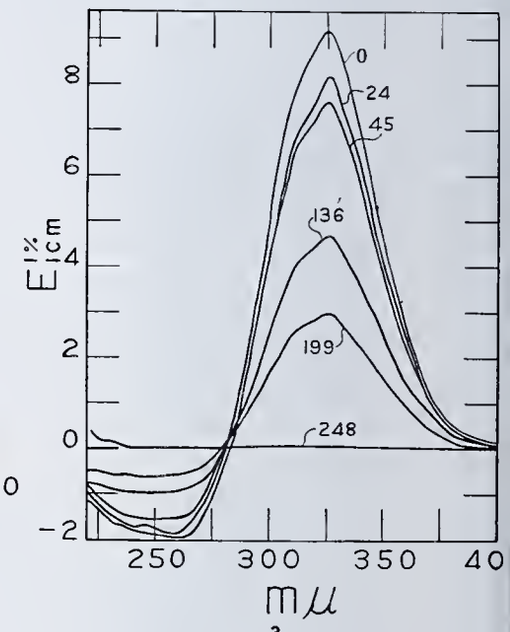

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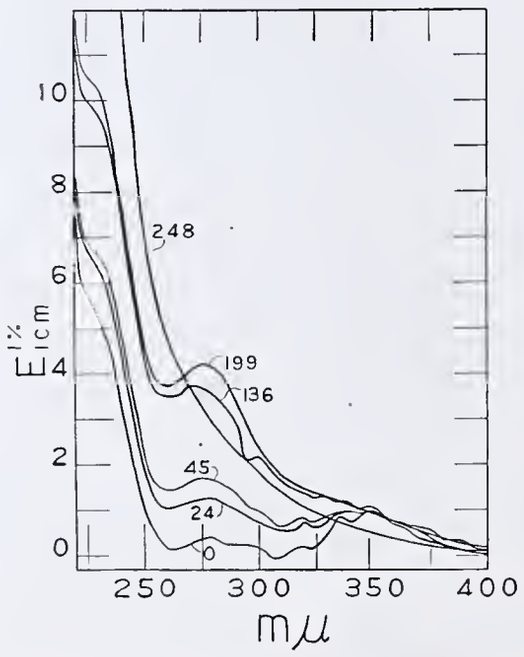

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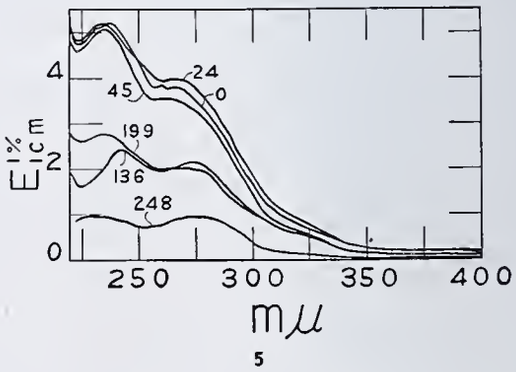

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Figures 1 to 5. Absorption Curves of Fractions of Grayfish Liver Oi Samples Oxidized at 75° C.

1. Whole oil
2. Unsaponifiable fraction
3. Modified values (unsaponifiable minus irradiated unsaponifiable)
4. Whole oil minus unsaponifiable (saponifiable carrier)
5. Irradiated unsaponifiable (unsaponifiable carrier)

Accelerated Oxidation at 75° C.

Vitamin A estimate[c,d]	Modified Values[a] Decrease in vitamin A estimate, %	Maximum at mμ	$E^{1\%}_{1\text{ cm.}}$ 300/328	$E^{1\%}_{1\text{ cm.}}$ 350/328	Per Cent Extraneous Absorption[b] Saponifiable Carrier At 328 mμ	At 280 mμ	At 260 mμ	Unsaponifiable carrier at 328 mμ	Total at 328 mμ
			Grayfish Liver Oil (Unsaponifiable 21.0%)						
18,060	0	325	0.57	0.51	1.6	20	19.2	7.8	9.4
16,100	10.9	325	0.53	0.51	5.0	28.4	34.8	8.4	13.4
14,980	17.0	325	0.56	0.52	7.3	41.0	41.7	7.1	14.4
9,220	49.0	325	0.55	0.54	18.7	63.0	77.7	9.2	27.9
5,900	67.4	325	0.58	0.54	25.8	67.9	71.4	10.8	36.6
140	99.2	...	0.82	0.69	84.8	75.5	85.9	8.5	93.3
			Ling Cod Liver Oil (Unsaponifiable 10.5%)						
61,280	0	325	0.48	0.55	5.5	16.1	12.9	5.5	11.0
58,280	4.9	325	0.50	0.54	9.0	17.9	23.5	5.0	14.0
49,280	19.6	326	0.49	0.56	13.9	34.4	41.5	9.5[e]	23.4[e]
40,400	34.1	326	0.50	0.57	19.2	39.0	44.1	7.6	26.8
23,980	60.9	325	0.55	0.55	19.6	54.6	60.0	8.7	28.3
13,400	78.1	325	0.52	0.55	25.1	69.4	73.5	10.5	35.6
940	98.5	315	1.00	0.58	82.8	87.3	86.6	7.7	89.5

[d] Values too low, owing to absorption of irradiated vitamin A.
[e] Value too high, owing to insufficient irradiation of sample.

The appearance of the new maxima during oxidation is generally attributed to the formation of different conjugated systems, produced by isomerization in the case of unconjugated fats and by the destruction of a part of the four conjugated double bonds in the case of vitamin A. Both components of the oxidized saponifiable carrier (fatty acid and oxidized vitamin A) can therefore be responsible in various degrees for the formation of the 275 mμ band. The extinction coefficients of conjugated fatty acids are of such a high order (*3*) that their formation even in small quantities can markedly affect the shape of the vitamin A curve.

It can be seen in Figures 1 and 6 that the whole left side of the vitamin A curve is affected by the formation of the three new maxima. The minimum of the vitamin A curve at 260 mμ steadily increases during oxidation despite loss in potency. This causes a broadening of the curve, an increase of the $E^{1\%}_{1\text{ cm.}}$ 300/328 ratio, and a shifting of the 328 mμ peak to a lower wave length in the later stages of oxidation. By studying the curves of the saponifiable carrier (Figures 4 and 9) it can be seen that the point at 260 mμ (minimum of vitamin A curve) or at about 280 mμ (maximum of the saponifiable carrier) is more indicative for taking the ratio than the 300 mμ point. At these points the $E^{1\%}_{1\text{ cm.}}$ values increase during oxidation, while at the same time $E^{1\%}_{1\text{ cm.}}$ values at 328 mμ decrease (Figures 1 and 6). At 300 mμ the $E^{1\%}_{1\text{ cm.}}$ values remain rather constant while those at 328 mμ decrease. The point at 300 mμ was apparently empirically selected not for any significance in the detection of extraneous absorption but for the convenience of taking readings at a wave length where the use of quartz cuvettes and of a hydrogen discharge lamp is unnecessary. In Figure 11 are plotted the ratios of $E^{1\%}_{1\text{ cm.}}$ 300/328, 280/328, and 260/328 of the oxidized test oils. The steeper curves of the 260 and 280 ratios are the graphical results of the considerations mentioned above.

The extraneous absorption (expressed in per cent of the absorption of the whole oil) of the saponifiable carrier steadily increases during oxidation and could also be used as a criterion for the evaluation of the quality of vitamin A oils. At 328 mμ the extraneous absorption of the saponifiable carrier increases only slightly during oxidation, the increase being dependent mostly on formation of the maxima at 275 to 280 mμ and at 335 to 345 mμ. The increase in per cent extraneous absorption is therefore mainly due to the decrease of vitamin A. Such an increase of per cent extraneous absorption at 328 mμ was also observed by Oser *et al.* (*18*) on different high-potency oils and by Coy *et al.* (*5*, *6*) on cod liver oils. It can be seen, however, in Figure 12, that the first stages of oxidation, which are important from the practical standpoint, result in only a slight increase and are possibly of little value as criteria. At 260 and 280 mμ the per cent of extraneous absorption increases significantly during the first stages of oxidation. It is most pronounced at 260 mμ because here the influence of the 235 mμ maximum is greater than at 280 mμ. Both points, however, could be used as criteria. A certain advantage can be given to the 260 mμ point, at which wave length the determination can be carried out more accurately on the flat of the curve.

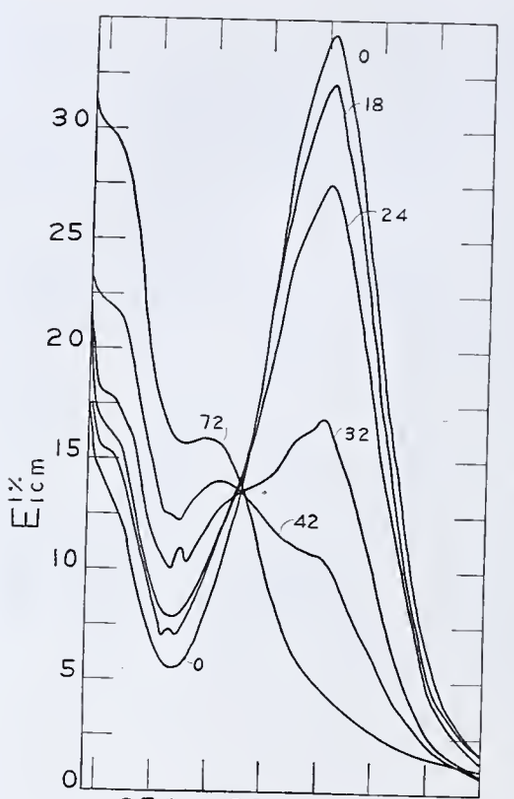
Unsaponifiable Carrier. It is obvious that vitamin A determinations should not be carried out on the whole oil. The determination on the unsaponifiable fractions will be sufficient in cases where the extraneous absorption of the saponifiable carrier does not change considerably during the first stages of oxidation. Commercially prepared oils which frequently are slightly oxidized would thus not give erroneously high absorption values at 325 mμ when determined on the unsaponifiable fractions and calculated with a uniform factor.

Curves showing essentially the absorption of the unsaponifiable carrier are represented in Figures 5 and 10. The vitamin A appears to be destroyed in all samples by irradiation of the unsaponifiable fraction, with the exception of the 18-hour sample of ling cod liver oil. It can be seen in the curves of both test oils that a new maximum at 270 mμ appeared during irradiation. It represents a substance formed from vitamin A during destructive irradiation. The formation of a similar maximum at 275 mμ during irradiation of vitamin A acetate was observed by Sobotka *et al.* (*21*). The absorption band of the irradiated unsaponifiable fraction includes, therefore, not only the absorption of substances originally present in the unsaponifiable fraction, but also that of the irradiation product of vitamin A.

The additional absorption of the irradiation product of vitamin A affects considerably the curves of the modified values (Figures 3 and 8) which, as mentioned above, are obtained by subtraction. The entire left side of the modified curves appears steeper and results in negative values below 280 mμ. Part of the absorption at 325 mμ can be caused by the absorption of the irradiation product which seems to extend beyond 325 mμ, with the result that the modified vitamin A value appears to be too low.

During oxidation of the test oils the absorption of the irradiated samples at 325 mμ showed a tendency to decrease but the differences were within the range of experimental error. It can therefore be assumed that no significant changes occur in the unsaponifiable carrier during the first stages of oxidation of either test oil at 325 mμ. The determination of the modified values is therefore not justified.

The unsaponifiable carrier of the ling cod liver oil (Figure 10) shows no pronounced maxima caused by components originally present in this fraction. The unsaponifiable carrier of grayfish liver oil (Figure 5), on the other hand, contains substances absorbing at about 238 mμ. The absorption of these substances partly masks the absorption of irradiated vitamin A at 270 mμ. The absorption at 238 mμ, however, does not affect the 325 mμ point. It remains constant in the first stages of oxidation, but decreases considerably later. In order to identify the substances, the unsaponifiable fraction of a grayfish liver oil was chromatographed, using Swain's method (*22*) of the flowing chromatogram. The absorption curves of three fractions obtained by elution with different solvents are presented in Figure 13. The amount of absorption of the separate fractions is expressed in $E^{1\%}_{1\text{ cm.}}$ values based on the weight of the total unsaponifiable fraction. The absorption curve for the vitamin A eluate is omitted in the figure. It can be seen that the main substance absorbing at 238 mμ belongs to the glyceryl ether fraction, prob-



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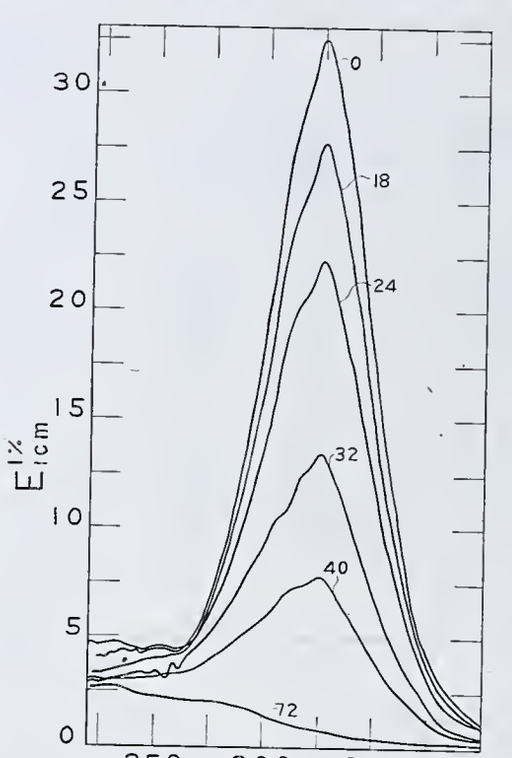

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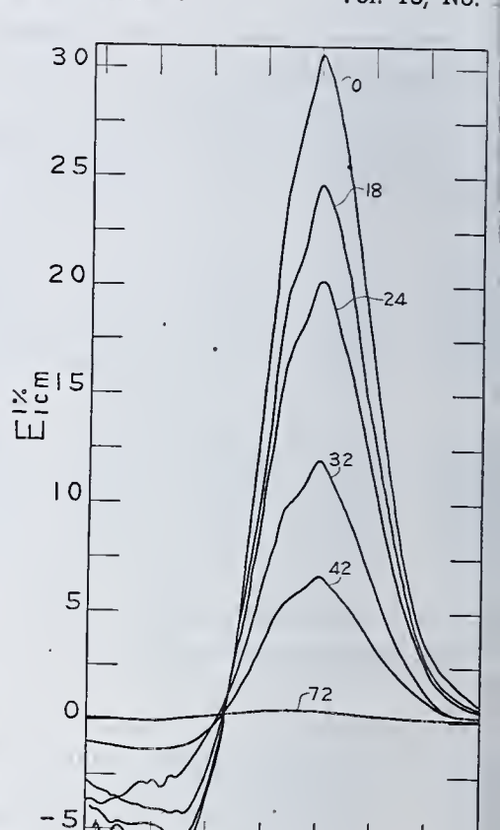

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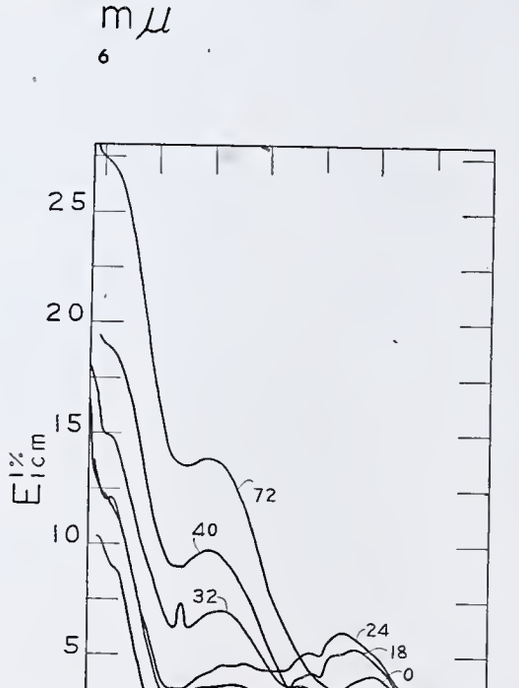

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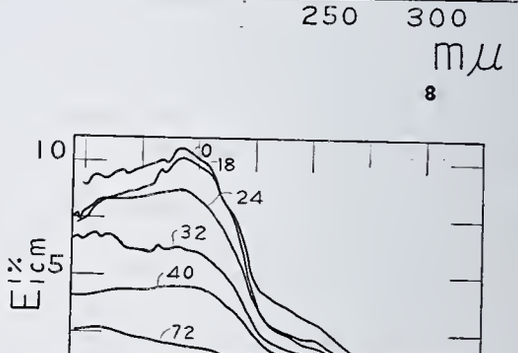

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Figures 6 to 10. Absorption Curves of Fractions of Ling Cod Liver Oil Samples Oxidized at 75° C.

6. Whole oil
7. Unsaponifiable fraction
8. Modified values (unsaponifiable minus irradiated unsaponifiable)
9. Whole oil minus unsaponifiable (saponifiable carrier)
10. Irradiated unsaponifiable (unsaponifiable carrier)

ably being a higher unsaturated homolog of selachyl alcohol (*22*). Another substance absorbing very strongly at 238 mμ was discovered in the methanol eluate. This substance of unknown constitution is present in only very small quantities.

Although the unsaponifiable carrier of the two test oils did not show oxidizable substances which would affect considerably the absorption curve of vitamin A, some fish liver oils can contain substances which carry through to the unsaponifiable fraction and produce an anomalous vitamin A curve. In presence of such substances as kitol (*10*), vitamin A_2 (*9*), anhydro vitamin A (*20*), and others, an accurate estimate of vitamin A by means mentioned above is impossible. The commercial conversion factor of 2000 allows a certain amount of extraneous absorption in all oils because the crystalline vitamin A alcohol was shown to have a conversion factor 2460 (*1*). In cases where the substances which produce excessive extraneous absorption in the unsaponifiable fraction do not interfere with the Carr-Price reaction, the colorimetric estimate can be used advantageously (*19*).

CONCLUSIONS

The ratio $E^{1\%}_{1\ cm.}$ 280/328 was found more indicative of the quality of fish liver oils containing vitamin A than the ratio $E^{1\%}_{1\ cm.}$ 300/328 since, while the 300 mμ point was arbitrarily selected,

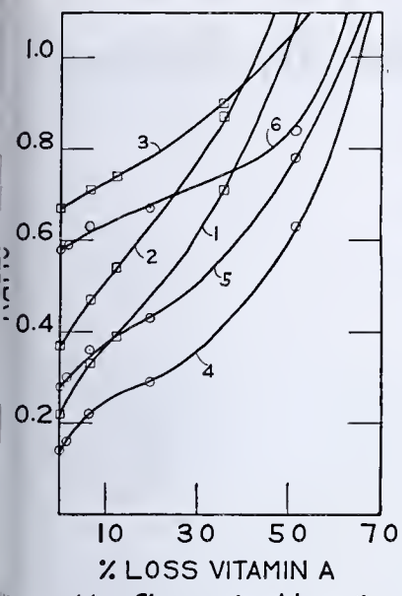

Figure 11. Changes in Absorption Ratios of Test Oil Samples Oxidized at 75° C.

1. Grayfish liver oil, ratio 260/328
2. Grayfish liver oil, ratio 280/328
3. Grayfish liver oil, ratio 300/328
4. Ling cod liver oil, ratio 260/328
5. Ling cod liver oil, ratio 280/328
6. Ling cod liver oil, ratio 300/328

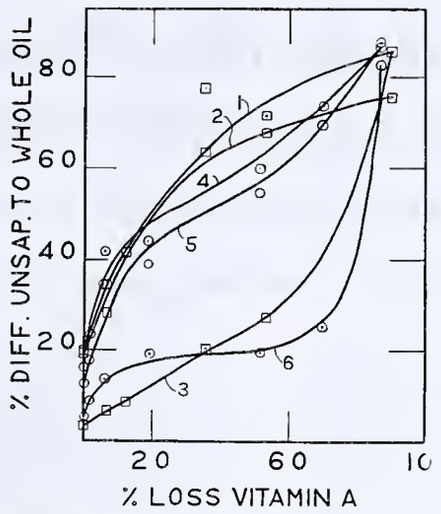

Figure 12. Per Cent Absorption of Saponifiable Carrier

$$\frac{\text{Whole oil, minus unsaponifiable}}{\text{whole oil}} \times 100$$

1. Grayfish liver oil at 260 mμ
2. Grayfish liver oil at 280 mμ
3. Grayfish liver oil at 328 mμ
4. Ling cod liver oil at 260 mμ
5. Ling cod liver oil at 280 mμ
6. Ling cod liver oil at 328 mμ

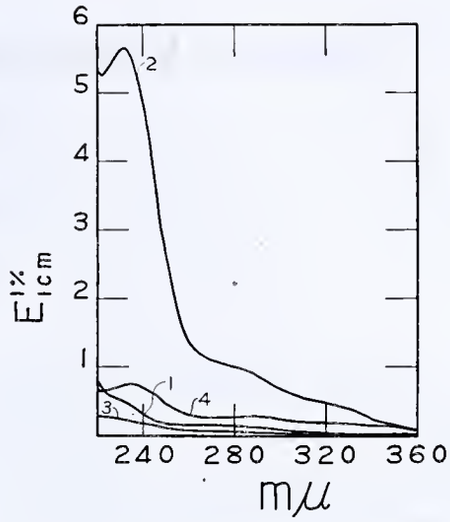

Figure 13. Absorption Curves of Fractions of Unsaponifiable Grayfish Liver Oil Obtained by Flowing Chromatography

$E^{1\%}_{1\text{ cm.}}$ values calculated on basis of total unsaponifiable

1. Petroleum ether, eluate, 1.4% (hydrocarbons)
2. Ether eluate, 81.4% (glyceryl ethers)
3. Chimyl alcohol, m.p. 59.5-50°, crystallized from ether eluate, 20.8%
4. Methanol eluate, 2.6%

Not shown, benzene eluate (vitamin A and sterols), 11.2%

e saponifiable carrier develops at 280 mμ a definite maximum uring oxidation. The formation of this maximum is responsible r the increase in the ratios on the left slope of the vitamin A urve in oxidized fish liver oils. The 280 mμ point shows therere a proportionately greater increase in its absorption value at e same degree of oxidation than the 300 mμ point. Alternavely, the ratio $E^{1\%}_{1\text{ cm.}}$ 260/328 could be used because at 260 mμ, e minimum in the vitamin A curve, the determination can carried out more accurately than on the slope. The 260 mμ oint is not only affected by the maximum at 280 mμ, but also the increased absorption at wave lengths lower than 260 mμ used by oxidation of the saponifiable carrier.

Considering the great difference in the ratios of both original st oils, it seems that the establishment of a definite limit for its agnitude for evaluating the quality of fish liver oils is not satisctory. A rigorous limitation for good oils and a range for doubtl ones would be more satisfactory. It is suggested that in doubtl cases the percentage of extraneous absorption of the saponible carrier $\dfrac{E^{1\%}_{1\text{ cm.}}\text{ whole oil} - E^{1\%}_{1\text{ cm.}}\text{ unsaponifiable}}{E^{1\%}_{1\text{ cm.}}\text{ whole oil}} \times 100$ at 30 or 260 mμ might be helpful in the estimation of the quality fish liver oils.

From the present work and from other experience in this laboratory it appears that the value of 0.72 for the $E^{1\%}_{1\text{ cm.}}$ 300/328 incates that oxidation has taken place, in the case of high-potency ls at least. Further work, particularly biological, will be necesry to establish proper limitations of the values to be used as iteria.

The irradiation technique for determining vitamin A was not und suitable for high-potency fish liver oils because the irraated vitamin A which has a maximum at about 275 mμ shows a rtain absorption at 325 mμ. This results in the modified abrption values at 325 mμ being too low.

In both test oils the absorption of the unsaponifiable carrier at 25 mμ was found to remain stable during oxidation. Taking to account the fact that the unsaponifiable fraction of grayfish ver oil is composed mostly of glyceryl ethers and that of ling cod ver oil of cholesterol, it appears that a fairly accurate estimation the quantity of vitamin A can be made in high-potency fish liver oils on the unsaponifiable fraction, even when the oils are oxidized.

ACKNOWLEDGMENT

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Indirect Calibration of the Filter Photometer by Means of the Spectrophotometer

Considerations on Use of Standards

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Absorption spectra are very sensitive to changes in molecular structure which may otherwise pass unnoticed, and serious errors occur in filter photometry and spectrophotometry unless the absorption coefficients of the standards are identical with those of the unknown in the measured solution. Changes in the absorption of the standard may result from standing or from chemical treatment during the isolation procedure. This situation occurs frequently, since standards are usually subject to a rigorous purification process whereas the analytical determinations are usually made on simple extracts in which the substance measured has had little opportunity to undergo degradation. This type of error can be avoided and the method simplified by use of appropriate calibration procedures which may involve interchangeability of spectrophotometric data.

COLORIMETRIC and spectrophotometric analytical methods may be broadly classified into two groups: those in which the substance or element is treated with a reagent to form a system having suitable colorimetric properties and those in which the substance itself has absorption characteristics suitable for the analytical measurement. By far the greater number of colorimetric methods fall into the first category, and adequate reviews have been published emphasizing the limitations and the precautions to be observed with this type of method (*7*, *8*). While comparatively few systems are found in the second group, many of the biologically important compounds, especially the complex substances of high molecular weight, have absorption spectra which allow measurement without the need for chemical transformations.

If the analytical results are to have any meaning, it is essential that the absorption spectrum of the standard be identical with that of the compound under consideration as measured in the unknown solution. This paper emphasizes the precautions which should be observed where absorption spectra of purified substances are used for analytical purposes, and more particularly where purified substances are used as calibration standards in filter photometry. Advantages of an indirect method, which involves calibration of the filter photometer by means of the spectrophotometer and established absorption coefficients, are presented. Although this work has been developed employing photoelectric colorimeters, the method should be applicable to visual instruments as well.

SPECTROPHOTOMETRIC METHODS

Within the past few years the availability, at reasonable cost, of commercial spectrophotometers capable of absolute absorption measurements has placed this facility at the disposal of a great many laboratories throughout the country. While the use of published absorption coefficients must always be approached with caution, it seems evident that if the physical and instrumental factors are satisfactorily taken into account, significant errors should not be introduced by using coefficients on instruments other than the one on which they were established (see *4* for definition of absorption coefficient). Methods are available by which the worker can determine for himself that his individual spectrophotometer is giving linear response, is in proper wavelength adjustment, and that significant amounts of scattered radiation are absent (*6*, *9*).

The size of the spectral region isolated is a factor which must be considered in terms of the substance being measured. Since any given measurement represents the integrated absorption over the spectral region isolated, it follows that an instrument capable of giving absolute absorption coefficients for a substance with a broad maximum may not be satisfactory for a substance with a narrow absorption peak. Table I (*2*) shows the effect of the spectral region isolated on the absorption coefficients of chlorophyll *A* in diethyl ether solution; these measurements were made with a photoelectric spectrophotometer at Purdue University similar to that described by Hogness, Zscheile, and Sidwell (*6*) but containing a Müller-Hilger double monochromator. It is clear that for absolute measurements on this particular system the spectral region isolated should not exceed 31 to 36 Å. at 6600 Å., or about 60 Å. at 4290 Å.; at the broad minimum near 4720 Å. significant errors are not introduced even when the region isolated approaches 155 Å. Zscheile *et al.* (*15*) have reported similar information for β-carotene. These data illustrate how this particular instrumental requirement for absolute measurements on a given system may be determined. The spectral region isolated for given slit settings on an instrument may usually be obtained from the manufacturer's specifications but should be checked by established methods (*6*).

It is axiomatic that a worker should determine absorption coefficients on his own instrument whenever possible. In the case of many of the biologically important substances, however, it is very difficult to obtain the isolated highly purified material with its absorption properties unaltered; in other words, differences between laboratories may more often be due to variations in the samples measured than to instrumental differences. It is in such instances that interchangeability of spectrophotometric data becomes of definite advantage. Zscheile, Comar, and Mackinney (*14*) exchanged samples and solutions of purified chlorophylls and inorganic salts (Weigert's solution) and made comparative measurements on the Purdue spectrophotometer mentioned above and another instrument described by Smith (*11*). There were no instrumental differences and it was evident that interchangeability of data on the systems studied was feasible. Comar (*3*) showed that absolute absorption coefficients for the chlorophyll components as determined on the Purdue spectrophotometer could be used with a Cenco-Sheard spectrophotelometer to give accurate analytical results. Vandenbelt, Forsyth,

Table I. Effect of Spectral Region Isolated on Absorption Coefficients of Chlorophyll *A* in Diethyl Ether Solution

Wave Length 6600 Å. (Maximum)		Wave Length 4290 Å. (Maximum)		Wave Length 4720 Å. (Minimum)	
Region isolated (Å.)	$\text{Log}_{10}\frac{I_0}{I}$	Region isolated (Å.)	$\text{Log}_{10}\frac{I_0}{I}$	Region isolated (Å.)	$\text{Log}_{10}\frac{I_0}{I}$
10	0.442	10	0.582	9	0.484
15	0.445	13	0.581	11	0.486
20	0.444	16	9.581	13	0.484
25	0.442	21	0.582	18	0.487
31	0.442	26	0.583	22	0.486
36	0.437	33	0.581	33	0.485
41	0.430	41	0.581	44	0.486
51	0.424	49	0.576	55	0.487
61	0.416	66	0.574	66	0.487
71	0.409	82	0.568	89	0.487
81	0.402	99	0.563	110	0.486
102	0.389	114	0.556	155	0.489
153	0.359	164	0.536	177	0.492

and Garrett (*12*) have demonstrated that a Beckman spectrophotometer can give absolute absorption coefficients with certain inorganic salts and the natural Vitamin A ester. It is obvious then that interchangeability of spectrophotometric data on a sound basis will soon be possible among many laboratories.

The sensitivity of the absorption spectra of complex organic compounds to changes in molecular structure is a disadvantage in analytical studies. Small modifications of the structure of high molecular weight compounds, such as changes in the degree of conjugation of double bonds or in the elements comprising the conjugated system, can scarcely be detected by ordinary chemical methods and yet they may profoundly influence the absorption characteristics. Therefore it should always be ascertained that the spectrum of the compound in question has not undergone any change during the isolation and purification procedure prior to determination of the absolute absorption coefficients. This can be done critically and conveniently by employing the absolute absorption coefficients at several wave lengths for the analysis of the simple extract. As an illustration, consider the chlorophyll components; it was necessary to demonstrate that the absorption of the chlorophylls after rigorous purification was identical with that of the pigments after immediate extraction of the leaf material with solvent. Comar and Zscheile (*5*) obtained analytical agreement between measurements at several wave lengths when simple plant extracts were analyzed spectrophotometrically using the established absorption coefficients, which was considered sufficient evidence for the validity of the original absorption data. The analytical results are extremely sensitive to changes in the absorption and satisfactory values are not obtained if there are significant discrepancies in the absorption data. This procedure may also be used to determine the adequacy of a spectrophotometer for a given compound when a purified preparation is unobtainable. For instance, if a simple plant extract were analyzed satisfactorily for the chlorophyll components at several wave lengths using established absorption coefficients, this would indicate that the particular spectrophotometer was adequate for absolute measurements on this system.

FILTER PHOTOMETRY

Whenever possible it is desirable that a spectrophotometric method be adapted for use with the photoelectric colorimeter. Practically every laboratory has a colorimeter available and this instrument is less complicated and more easily used than the spectrophotometer. Absolute absorption measurements are not possible with today's photoelectric colorimeter and each instrument must have its own calibration curve for each substance. Interchangeability of calibration and absorption data is not to be recommended even between instruments of the same make and design.

The filter photometer is usually calibrated by using weighed amounts of the purified compound and making up solutions of known concentration. Some function of the absorption of each solution is plotted against the concentration to give the calibration curve. In the case of labile substances, this method may produce errors which pass unnoticed and are therefore all the more serious. This situation arises because the substance used for the calibration standard has usually been exposed to conditions during isolation, purification, and storage which may cause changes in the absorption, whereas the analytical measurements are customarily made on simple extracts in which absorption changes are at a minimum. This type of error is illustrated below.

A practical solution to this problem, where absorption coefficients have been established, involves calibration of the photoelectric colorimeter by means of spectrophotometric measurements on the simple extract. This method has proved entirely satisfactory for chlorophyll analysis (*1*, *4*). The general procedure is as follows:

The worker prepares a simple extract in the manner decided upon for the colorimetric analysis. From the original extract a series of known dilutions is made which covers the optimum $\log_{10} \frac{I_0}{I}$ range of the colorimeter to be used. The absorbency of each dilution is measured on the colorimeter, and an aliquot of the original extract is measured on a spectrophotometer which is known to be satisfactory for absolute measurements on the system under study. The concentration of each of the dilutions used for the colorimetric measurement can then be calculated from the spectrophotometric value, and the calibration curve constructed.

Table II (see *4* for details) shows the results obtained with chlorophyll by this method and also points out the deviations which resulted when various purified preparations were used as calibration standards. The spectrophotometric as well as the colorimetric measurements were made on aliquots from the same leaf extract. These data support the viewpoint that colorimetric results must be viewed with suspicion where the calibration standard has not been studied spectrophotometrically.

Table III shows how this indirect method of calibration may be employed for the determination of total carotene.

In this study a Fisher Electrophotometer was used with 23-ml. cylindrical cells, and the 425 filter supplied with the instrument. The data for the direct calibration were obtained in the usual way by using weighed amounts of crystalline carotene (90% β, 10% α, from S.M.A. Corporation) in hexane. This sample of carotene had been stored under vacuum, in the dark, at refrigerator temperature until used. For the indirect calibration a plant extract was prepared from fresh carrots according to the method

Table II. Per Cent Deviation from Spectrophotometric Value for Total Chlorophyll

Plant Leaf Material	Photoelectric Colorimeter Calibrated with Chlorophyll in plant extract	Chlorophyll sample Z.-C.[a]	Chlorophyll sample 1[b]	Chlorophyll sample 2[c]
Norway maple	3.5	22.6	35.2	75.9
Muskmelon	1.0	21.1	37.7	82.9
Alfalfa	−3.9	14.7	30.0	70.4
Crimean linden	1.8	23.0	35.0	76.7
Tomato	0	20.1	37.3	78.1
Lima bean	−3.7	13.3	32.1	69.7
Watermelon	9.8	30.8	49.0	94.4
Peanut	−4.8	14.8	29.2	72.2
Bush string bean	−1.6	12.0	39.1	67.4
Pepper	−1.0	12.8	36.0	67.0
Oats	−3.5	16.3	32.2	71.3
Wheat	−2.0	18.3	33.7	74.8
Broccoli	−6.2	13.8	27.5	69.2
Spinach	−4.0	16.0	32.0	70.4
Beets	−2.7	19.1	41.8	72.7
Carrot	−3.3	16.7	32.5	73.2
Blue grass	−0.8	18.5	34.3	74.6
Sunflower	−4.8	14.3	31.2	72.5
Rhubarb	0	20.3	36.7	77.2
Black locust	−7.3	13.3	27.6	67.3
Hollyhock	−4.2	15.7	31.5	72.7
Peach	10.0	32.4	50.7	99.1
Clover	−2.2	16.4	30.3	69.3
Burdock	−4.3	14.7	32.2	73.9

[a] Prepared by method of Zscheile and Comar (*13*), absorption coefficient at 6600 Å. in diethyl ether 83 liters per gram cm.
[b] Research grade commercial chlorophyll, absorption coefficient at 6600 Å. in diethyl ether 44 liters per gram cm.
[c] Research grade commercial chlorophyll, absorption coefficient at 6600 Å. in diethyl ether 33 liters per gram cm.

Table III. Data for Calibration of Photoelectric Colorimeter for Carotene Analyses

(Direct calibration using crystalline carotene compared with indirect method using spectrophotometric determination on plant extract)

Direct Calibration with Crystalline Carotene		Indirect Calibration with Plant Extract	
Carotene concentration	$\log_{10} \frac{I_0}{I}$	Carotene concentration	$\log_{10} \frac{I_0}{I}$
Mg./l.		*Mg./l.*	
4.73	0.802	4.53	0.796
3.15	0.695	3.02	0.675
2.10	0.552	2.01	0.529
1.40	0.417	1.34	0.412
0.93	0.312	0.90	0.296
0.62	0.207	0.60	0.205
0.41	0.140	0.40	0.143

of Moore (*10*). Aliquots of this extract were measured on the colorimeter and the carotene concentrations were determined by measurements at 4520 Å. on a Beckman spectrophotometer, using the absorption coefficients as reported for pure carotenes in hexane by Zscheile *et al.* (*15*). The slit width was 0.03 mm. and 1-cm. cells were employed.

These data, when plotted, give essentially the same calibration curve. This indicates that when a good preparation of carotene is used, the analytical results will be the same with either the direct or indirect method of calibration. Any discrepancy in a comparison of this type will probably be due to deterioration of the crystalline carotene standard, so that the indirect calibration curve is considered the more reliable.

ADVANTAGES OF INDIRECT CALIBRATION

The fundamental advantage of the indirect method of calibration as outlined here is the reliability of the results obtained, which will lead to dependable interlaboratory agreements where this situation is now far from satisfactory in many cases. Another advantage lies in the simplicity of the indirect method. After the details have been worked out, the spectrophotometric determination on an aliquot of the extract is usually a simple matter of making measurements on a solution at a few given wave lengths. It can usually be arranged to have the solutions shipped to another laboratory for the spectrophotometric measurement, if necessary. This practice eliminates the need for obtaining the purified material either by purchase or preparation and for its carefully controlled storage, and eliminates the operation of weighing out the standard and the necessity for spectrophotometric study of the standard prior to each use.

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Determination of Conjugated Diolefins with Chloromaleic Anhydride

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A new method of determining conjugated diolefins in hydrocarbon mixtures utilizes chloromaleic anhydride as the dienophilic reagent. This method is based on the quantitative addition of chloromaleic anhydride to conjugated dienes to form an adduct containing a highly reactive tertiary chlorine atom which can be determined by the Volhard method after refluxing with aqueous silver nitrate; the vinyl chlorine of the reagent is completely unreactive under the same conditions.

DURING the early phases of the synthetic rubber program, an investigation of the preparation of conjugated dienoid monomers by the cracking of terpenes was undertaken in this laboratory. It soon developed that the determination of conjugated dienes in the pyrolysis products was one of the more important phases of the problem. Since no satisfactory procedure was available for the analysis of such mixtures, a number of physical and chemical methods were considered.

It was recognized that several physical methods including ultraviolet absorption, infrared absorption, and mass spectrometry give excellent results on mixtures of known constituents. However, to obtain reliable results by the absorption methods it is generally necessary to know not only the functional groups but also the molecular species present. Moreover, a simple chemical method requiring no special equipment appeared desirable for the routine determination of conjugated dienes in the crude products from cracking experiments. Consequently, a number of reagents were considered which included bromine (*4, 7*), sulfur dioxide (*10, 12*), aromatic diazonium coumpounds (*11*), cuprous chloride (*9*), maleic anhydride (*3, 12*), and chloromaleic anhydride. All these reagents except the last two either proved nonspecific for conjugated dienes or did not react quantitatively.

The first procedure developed involved fractionation of the samples and determination of the conjugated diene content of the cuts by a refined gas volumetric procedure utilizing maleic anhydride as the diene absorbent. However, the need for a more rapid procedure—particularly one that would not involve preliminary fractionation of the sample—resulted in the development of a second method using the same reagent. In this procedure the conjugated diene content was obtained by reacting the sample with a known excess of maleic anhydride and determining the unreacted reagent polarographically. Both procedures were time-consuming and provided only an indirect determination of the conjugated dienes.

The belief that chloromaleic anhydride would also react quantitatively with conjugated dienes and, in addition, would provide a direct and more rapid determination led to the development of the present method.

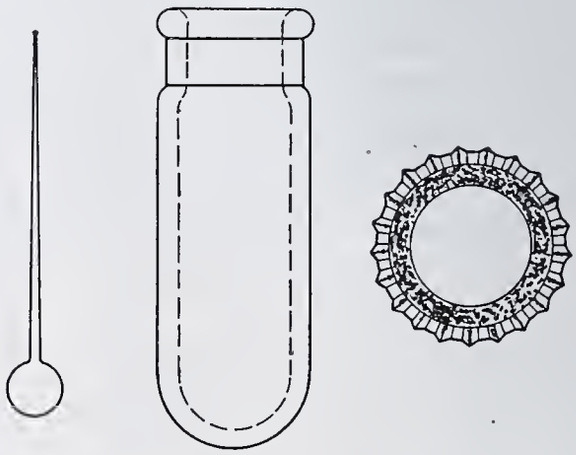


Figure 1. Sample Bulb, Pressure Bottle, and Bottle Cap

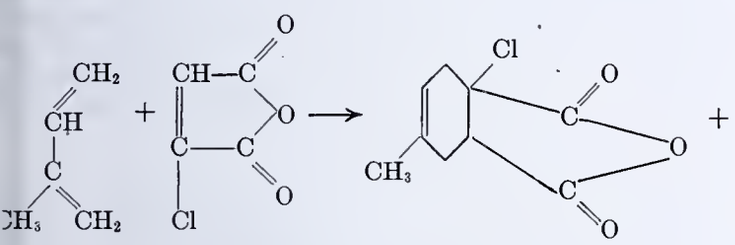
Table I. Determination of Chlorine in Isoprene–Chloromaleic Anhydride Adduct[a]

Compounds Added	Conditions of Pretreatment: Temp., ° C.	Conditions of Pretreatment: Time, hours	Chlorine in Adduct: Theoretical, %	Chlorine in Adduct: Found, %
None	...	...	17.68	17.7
None	...	...	...	17.6
None	...	...	...	17.6
Chloromaleic anhydride, 1 gram	55	2	...	17.9
Chloromaleic anhydride, 1 gram	50–55	2.5	...	17.8
Mixed Amylenes, 0.2 ml.	50–55	2.5	...	17.7
Chloromaleic anhydride, 1 gram	100	2	...	17.7
	100	2	...	17.7
Inhibitor solution, 2 drops	75–80	1	...	17.8
	75–80	1	...	17.8

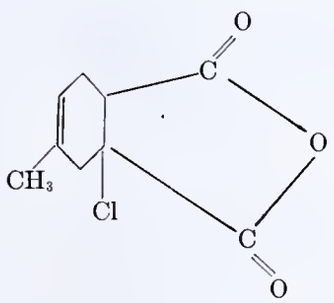
[a] Sample weights varied from 0.2 to 0.5 gram.

DEVELOPMENT OF CHLOROMALEIC ANHYDRIDE METHOD

Chloromaleic anhydride reacts with conjugated diolefins in the same manner as maleic anhydride to form a Diels-Alder adduct. Thus, isoprene (I) reacts with chloromaleic anhydride (II) to give 1-chloro-4-methyl-4-cyclohexene-1,2-dicarboxylic anhydride (III) and 2-chloro-4-methyl-4-cyclohexene-1,2-dicarboxylic anhydride (IV).



I II III



IV

The difference in reactivity between the highly active tertiary chlorine of the adduct and the unreactive vinyl chlorine of the reagent permits a quantitative determination of the adduct in the presence of excess reagent. Refluxing with aqueous silver nitrate removes the chlorine completely from the adduct, leaving the chlorine of the chloromaleic anhydride untouched.

It is believed that the utilization of the high reactivity of tertiary halogens as a basis of organic analysis is a principle that has considerable possibilities and has been exploited to only a limited extent. This same principle has been applied recently in the authors' laboratories to the determination of α,p-dimethylstyrene in styrene mixtures (*5*).

The practicality of the new method was demonstrated by substituting chloromaleic anhydride for maleic anhydride in the gasometric absorption procedure, and also by synthesizing pure isoprene–chloromaleic anhydride adduct (melting point 35–36° C.) and showing that the chlorine of the adduct could be determined by the Volhard method after refluxing with aqueous silver nitrate. As the results in Table I show, the agreement with theoretical is equally good when the determination is made in the presence of compounds added to simulate conditions of an actual analysis.

Samples containing conjugated dienes were then heated with excess chloromaleic anhydride in specially designed pressure bottles (*6*) (Figure 1), and the amount of adduct formed was determined as outlined above. It was soon apparent, as is shown by the data in Table II, that it was necessary to purify the commercial-grade chloromaleic anhydride to obtain quantitative adduct formation. Chloromaleic acid proved to be one of the most troublesome impurities, as it is readily formed by hydrolysis of the anhydride. The low and erratic results obtained in the presence of appreciable amounts of this acid, shown in Table III, may be due to the acid catalyzing the dimerization and polymerization of conjugated dienes, thus rendering them unavailable for adduct formation. The formation of chloromaleic acid can, however, be prevented by proper care in handling the anhydride.

Even with extensive purification it was difficult to obtain chloromaleic anhydride that gave theoretical adduct formation. Consequently, it was found desirable to use chloromaleic anhydride that gave results between 95 and 100% of theory and apply an empirical correction factor determined by analyzing a sample of known conjugated diene content. However, it is believed that if absolutely pure chloromaleic anhydride were available, it would be possible to eliminate the correction factor, since in one instance it has been reduced to 1.005.

Low and variable results were also obtained in the presence of peroxides which catalyze the copolymerization of conjugated dienes with chloromaleic anhydride. Apparently the copolymer contains more than a 1 to 1 ratio of conjugated diene to chloromaleic anhydride, and furthermore, it is so insoluble in aqueous silver nitrate that the analysis is unsatisfactory. In view of this adverse effect of natural peroxides, a small amount of inhibitor, such as *p-tert*-butylcatechol, was added as a preeaution in all subsequent analyses.

A study of the optimum time and temperature for adduct formation showed that heating at 55° C. for 2 hours was sufficient for samples containing over 25% isoprene or for cyclopentadiene. Samples containing smaller amounts of isoprene should be heated at higher temperatures and/or for longer periods of time—for example, samples containing 5 to 10% should be heated for 4 hours at 75° C. Butadiene reacts more slowly than isoprene and it is necessary to heat the samples at 55° C. for 12 hours to obtain satisfactory results. Isoprene-butadiene mixtures containing 5 to 10% butadiene require 6 to 8 hours at 55° C. and mixtures containing 20 to 25% butadiene require 8 to 12 hours at 55° C. The method should be applicable to samples containing less than 5% diene, but the optimum reaction conditions have not been established experimentally. The accuracy in this range might be improved by increasing the sample weight.

CHLOROMALEIC ANHYDRIDE REAGENT

Commercial chloromaleic anhydride is purified by filtering the crystals from the oily mother liquor and washing them with dry hexane. (Commercial chloromaleic anhydride may be pur-

Table II. Effect of Purity of Chloromaleic Anhydride on Determination of Conjugated Dienes

Chloromaleic Anhydride: Sample purification	Chloromaleic Anhydride: Melting point, ° C.	Conjugated Diene, Analysis Results, % of Theory
Commercial	27 (ca.)	62.2 ± 5
One recrystallization	31.5–32.5	91.6 ± 2
Two recrystallizations	32–34	93.1 ± 1
Three recrystallizations	32.5–34	95.4 ± 1
Two recrystallizations, one distillation	...	99.0 ± 1
Theoretical	33 (*1*) 34.5 (*8*)	100.0

Table III. Effect of Chloromaleic Acid on Determination of Conjugated Dienes with Chloromaleic Anhydride

Chloromaleic Acid Added, Weight % of Total Reagents	Weight % of Isoprene Found (Av.)
0	93.9
1	90.6
2	92.0
3	91.5
10	85.3
50	82.2

Table IV. Analysis of Standard Conjugated Diene Samples with Chloromaleic Anhydride

Composition of Sample	Conjugated Dienes, Weight % Present	Found[a]	Deviation
Isoprene	100.0	99.7	−0.3
	100.0	99.7	−0.3
	100.0	99.7	−0.3
	100.0	100.0	±0.0
	100.0	100.1	±0.1
Isoprene-amylene mixture	75.5	75.2	−0.3
	75.5	75.1	−0.4
	60.8	60.6	−0.2
	60.8	61.2	+0.4
	60.8	59.6	−1.2
	60.8	61.8	+1.0
	60.8	59.7	−1.1
	25.7	25.2	−0.5
	25.7	26.2	+0.5
	25.7	25.4	−0.3
Isoprene-pentane mixture	9.9	9.5	−0.4
	9.9	9.9	±0.0
	9.9	10.2	+0.3
	9.9	9.7	−0.2
	5.0	4.6	−0.4
	5.0	4.8	−0.2
Cyclopentadiene	98.9	97.5	−1.4
	98.9	98.5	−0.4
Butadiene[b]	98.4	97.8	−0.6
	98.4	97.9	−0.5

[a] Results corrected by a factor of 1.03.
[b] Butadiene samples heated for 12 hours at 55° C. instead of 2 hours.

Table V. Precision of Typical Determinations on Wide Boiling Range Isoprene Samples by Chloromaleic Anhydride Method

Sample Designation	Weight % of Conjugated Dienes Found, Calculated as C_5H_8
A	89.6, 89.7
B	74.4, 75.0
C	77.7, 77.5
D	85.3, 86.0
E	92.5, 92.8
F	95.4, 95.3
G	95.9, 96.0
H	91.6, 91.0
I	28.2, 27.3
J	93.8, 94.0

chased from the National Aniline Division, Allied Chemical and Dye Corporation, 40 Rector St., New York, N. Y. By special request one sample of chloromaleic anhydride was obtained which was of satisfactory analytical grade as received. Should such a reagent become generally available, the purification procedure would be unnecessary.) The crystalline chloromaleic anhydride is dissolved in the minimum amount of dry benzene and then hexane is added to incipient turbidity at room temperature. The mixture is seeded and cooled in an ice bath or a refrigerated room (+3° C.). The recrystallized product is filtered as before and then distilled in vacuo (water pump with drying tube in the line) under dry carbon dioxide or nitrogen. The fraction boiling at 110° C. at 44 mm. of mercury is collected and sealed in small glass ampoules under nitrogen or carbon dioxide until ready for use. The purified product melts at 32° to 34° C. and is completely soluble in dry benzene (insoluble material is usually chloromaleic acid). The correction factor for the reagent is determined by analyzing a sample of isoprene of known purity. If it is greater than 1.05, further purification is recommended.

ANALYTICAL PROCEDURE

For the analysis of volatile samples weigh a small glass bulb (Figure 1) similar to those used in acid analysis to the nearest 0.1 mg. Warm the weighed bulb slightly with a small flame and invert the stem into the sample, using a lead washer for support. Cool the bulb with a snugly fitting piece of dry ice until the desired amount of sample (0.1 to 0.2 gram) is drawn into it and then quickly seal the stem in a hot flame. To prevent carbonization and fractionation during sealing, keep the bulb well cooled by holding a small piece of dry ice just below the tip of the stem. Reweigh the bulb after it reaches room temperature and place it in a pressure bottle (Figure 1) with 1.0 ± 0.1 gram of chloromaleic anhydride. [These small pressure bottles, for use with commercial bottle caps, are available from the Ace Glass Co., Vineland, New Jersey. Bottle caps with cellophane liners ("spots") are recommended.] Add one drop (approximately 0.02 ml.) of a 10% solution of *p-tert*-butylcatechol in nitrobenzene, cap the pressure bottle using a household bottle capper, and break the sample bulb by striking the bottle sharply against a moderately hard object like the heel of one's shoe.

Heat for 2 hours at 55° C., cool, open the bottle, and transfer the reaction products to a 250-ml. Erlenmeyer flask through a funnel with 10 ml. of acetone and distilled water (50 to 75 ml.). (These conditions are for samples containing over 25% isoprene. See the preceding discussion regarding the conditions to use for other samples.) Use 2 ml. of the acetone to rinse the bottle cap. Add 20 ml. of 0.2 N aqueous silver nitrate solution and reflux the mixture for 1 hour. After cooling, filter, wash the precipitate thoroughly with distilled water, add 5 ml. of 1 to 1 nitric acid and 1 ml. of ferric alum indicator to the filtrate, and titrate the excess silver nitrate with 0.1 N potassium thiocyanate solution.

Nonvolatile samples may be weighed directly into the pressure bottle from a small weight buret. From this point the procedure is the same.

Calculate the results as follows (for unknown samples an approximate molecular weight may be assumed or the results may be expressed as diene numbers):

$$\% \text{ conjugated diene} = [(\text{ml. of } AgNO_3 \times N\ AgNO_3) - (\text{ml. of KSCN} \times N\ \text{KSCN})] \times \left[\frac{\text{mol. wt. of diene} \times \text{correction factor}}{\text{wt. of sample} \times 10}\right]$$

DISCUSSION OF RESULTS

The conjugated diene content of a number of standard samples of isoprene, isoprene-amylene mixtures, isoprene-pentane mixtures, cyclopentadiene, and butadiene was determined by the chloromaleic anhydride method. The results given in Table IV indicate that the average error of the determination expressed as per cent conjugated diene is about 0.5 unit. Duplicate determinations on a number of crude isoprene samples are given in Table V and show the precision of the method to be of the same order of magnitude as the accuracy.

Styrene interferes in the determination because of the formation of a copolymer with chloromaleic anhydride, even in the absence of peroxide catalysts. The copolymer naturally contains tertiary chlorine which is determined along with the chlorine of the conjugated diene-chloromaleic adduct.

Although the method has been used most extensively for the determination of isoprene, it has been applied successfully to the determination of butadiene, cyclopentadiene, and other conjugated dienes. Preliminary experiments indicate that *trans*-piperylene and 2-methyl-1,3-pentadiene can be determined with chloromaleic anhydride, although 4-methyl-1,3-pentadiene cannot. The behavior of the latter compound is not surprising, since it has been found (*2*) that it will not form a Diels-Alder adduct with maleic anhydride. It is probable that the method could be applied to other conjugated dienes by varying the conditions of adduct formation to obtain a quantitative reaction.

ACKNOWLEDGMENTS

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Indicator Properties of Derivatives of 4′-Nitrophenylazo-1-naphthol

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Three new indicators of the 2-(4′-nitrophenylazo)-1-naphthol class are described and indicator properties given.

CONGO red indicator test paper, in common use in the dyestuff industry, possesses the practical advantage of changing from the normally red neutral form through a series of grays and blue-blacks to the blue acid form. With experience, changes in pH of 0.2 may be detected. During some experiments on the properties of 1-naphthol derivatives, the author observed that the monoazo dye, resulting from coupling *p*-nitrophenyl diazonium chloride to 1-naphthol-4,8-disulfonic acid, had indicator properties similar to Congo red but was acid- instead of alkali-sensitive. Analogous compounds prepared from derivatives of 1-naphthol and *p*-nitroaniline had similar properties, changing from the normal red neutral form to the blue alkaline form over various pH ranges and hence were useful in testing common alkaline mixtures—e.g., sodium bicarbonate and carbonate.

Hewitt (*3*) has described the indicator 4-(4′-nitro-3′-sulfophenylazo)-1-naphthol which changes from yellow (acid) to purple (alkali). Wenker (*6*) examined various 2,4-dinitrophenylazo-1-naphthol sulfonic acids but concluded that the dyes resulting from ortho coupling were lacking in sensitivity to pH changes. This paper describes the indicator properties of three typical dyes made by coupling 4-nitrophenyl diazonium chloride (or the 2-methoxy derivative) to 1-naphthol-3,8- or 4,8-disulfonic acids which permit of only ortho coupling.

EXPERIMENTAL

I. Disodium Salt of 2-(4′-Nitrophenylazo)-1-naphthol-4,8-disulfonic Acid. Alpha blue. A mixture of 138 grams (1 mole) of *p*-nitraniline and 72 grams of technical (95%) sodium nitrite in 1 liter of water was slowly added to an agitated mixture of 1000 grams of ice, 250 ml. of commercial 20° Bé. hydrochloric acid, and 2 grams of sodium nitrite. After 2 hours the resultant diazonium chloride solution was filtered to remove sludge and slowly added to a solution of 1 mole of 1-naphthol-4,8-disulfonic acid (*4*), 370 grams of sodium carbonate, and 1000 grams of ice in 1.5 liters of water. The combination was allowed to agitate overnight and was then filtered, reslurried in 3 liters of 10% sodium chloride solution, filtered, and dried at 60° C. in an atmospheric oven.

Pure coal-tar dye (as disodium salt) by titration with titanous chloride, 67.1%.

II. Disodium Salt of 2-(4′-Nitrophenylazo)-1-naphthol-3,8-disulfonic Acid. Epsilon blue. One mole of *p*-nitraniline was diazotized as above and then slowly added to a solution of 1 mole of 1-naphthol-3,8-disulfonic acid (*2*), 336 grams of sodium carbonate, and 1000 grams of ice in 2 liters of water. The resultant dye was isolated and dried as above.

Pure coal-tar dye (as disodium salt) by titration with titanous chloride, 93.7%.

III. Disodium Salt of 2-(2′-Methoxy-4′-nitrophenylazo)-1-naphthol-4,8-disulfonic Acid. Nitroanisole blue. A solution of 74 grams of 95% sodium nitrite in 150 ml. of water was added slowly to a mixture of 168 grams (1 mole) of 5-nitro-2-aminoanisole (E. I. du Pont de Nemours & Co.), 250 ml. of 20° Bé. hydrochloric acid, and 1000 grams of ice. After 2 hours' agitation, the diazonium chloride solution was filtered and

Table I. Wave Lengths and Indicator Exponents

	I	II	III
Wave length $\left(\frac{\Delta \log D}{\Delta \text{pH}} = \text{max.}\right)$, mμ	610	640	630
pK	9.6	12.0	10.0

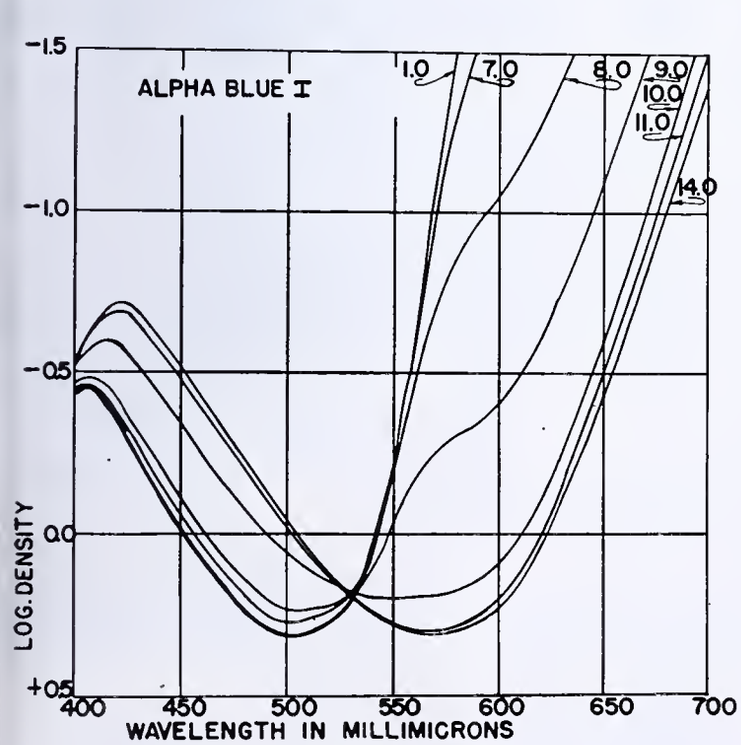

Figure 1

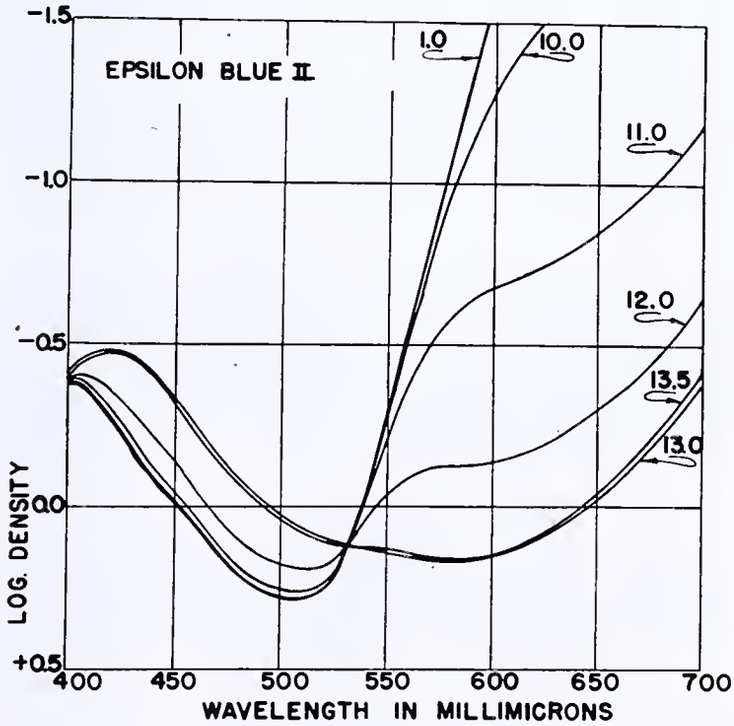

Figure 2

coupled to 1-naphthol-4,8-disulfonic acid in the same manner as for I.

Pure coal-tar dye (as disodium salt) by titration with titanous chloride, 74.1%.

INDICATOR PROPERTIES

Light Absorption. Spectral transmission curves (Figures 1, 2, and 3) of buffer solutions containing 10 p.p.m. of dry dye were obtained by means of the General Electric recording spectrophotometer, using a path length of 50 mm., band width of 10 mμ, temperature of 30° C., and distilled water as reference solvent. Buffer solutions for pH from 1 to 8 were essentially as described by Clark and Lubs (*1*); for pH 9, 10, 11, and 12, mixtures of sodium carbonate and bicarbonate were prepared; and for pH 13 and 14 solutions of sodium and potassium hydroxides were employed. All buffer solutions were checked by a Beckman pH meter just prior to determining the transmission curves. The presence of boric acid in Clark and Lubs buffers pH 9 and 10 was found to interfere with the dissolved dye.

Isosbestic points (*5*) in the visible region were at 531 mμ for I, 532 mμ for II, and 551 mμ for III.

INDICATOR EXPONENTS

Indicator exponents (pK) were determined graphically by observing the inflection points of the curves obtained by plotting optical density *vs.* pH. The density, D, was determined from the spectral transmission curves (Figures 1, 2, and 3) at the wave lengths of maximum difference of log density per unit change in pH—i.e., when $\frac{\Delta \log D}{\Delta \text{pH}} = \text{max.}$

Selected wave lengths and resultant indicator exponents are given in Table I.

PROPERTIES OF SOLUTIONS

Dilute aqueous solutions (10 p.p.m.) showed only small changes in transmittance after storage for one month in clear glass bottles exposed to artificial and diffused daylight but protected from direct sunlight.

Neutral aqueous solutions containing 0.10 gram of dry dye per 100 ml. were tested by adding 3 drops of indicator solution to 100 ml. of the reagents described in Table II and noting the resulting color.

PROPERTIES OF TEST PAPERS

Strips of Whatman No. 1 filter paper were immersed in a solution of 4.0 grams of dry dye in 1 liter of distilled water and allowed to drain and dry. These indicator papers were then tested by spotting with the various reagents with results as shown in Table II. The closest matching chip from the abridged Munsell Book of Color (Munsell Color Co., Inc., Baltimore, Md.) is given as the color.

DISCUSSION

Although all three indicators contain considerable amounts of inorganic salts and hence should be further purified for use in unbuffered media, the sharpness of the isosbestic points indicates that the organic purities are adequate for most purposes.

Alpha blue (I) appears to be particularly useful for distinguishing between bicarbonate and carbonate alkalinity, especially when used in the form of test paper.

Table II. Properties of Solutions and Test Papers

Reagent	Color of Solutions			Color of Test Paper		
	I	II	III	I	II	III
Distilled water	Pink	Pink	Pink	5R 6/10	5R 5/12	5R 5/12
Alcohol	Pink	Pink	Pink	5R 6/10	5R 5/12	5R 5/12
1 *N* HCl	Pink	Pink	Pink	5R 6/10	5R 5/12	5R 5/12
0.1 *N* HCl	Pink	Pink	Pink	5R 6/10	5R 5/12	5R 5/12
5% acetic acid	Pink	Pink	Pink	5R 6/10	5R 5/12	5R 5/12
5% sodium bicarbonate	Pink	Pink	Pink	5R 6/10	5R 5/12	10RP 5/10
5% $Na_2HPO_4.12H_2O$	Pink	Pink	Pink	10RP 4/10	5R 5/12	5R 5/12
5% sodium carbonate	Light purple	Purplish pink	Light purple	10PB 3/8	10R 4/8	10PB 3/8
5% $Na_3PO_4.12H_2O$	Light purple	Pale bluish purple	Light purple	10PB 3/10	5R 3/4	10PB 3/10
0.1 *N* NaOH	Light purple	Pale bluish purple	Light purple	10PB 3/8	10PB 3/8	10PB 3/8
Buffer, pH						
8	Pink	Pink	Pink	5R 6/10	5R 5/12	5R 5/12
9	Purplish pink	Pink	Pink	2.5RP 3/4	5R 5/12	10RP 4/10
10	Light purple	Pink	Purplish pink	5P 3/4	5R 5/12	2.5RP 4/6
11	Light purple	Pink	Light purple	10PB 3/8	5R 5/12	10PB 3/8
12	Light purple	Purplish pink	Light purple	10PB 3/8	10R 4/8	10PB 3/8
13	Light purple	Light purple	Light purple	10PB 4/10	10PB 3/8	10PB 4/8

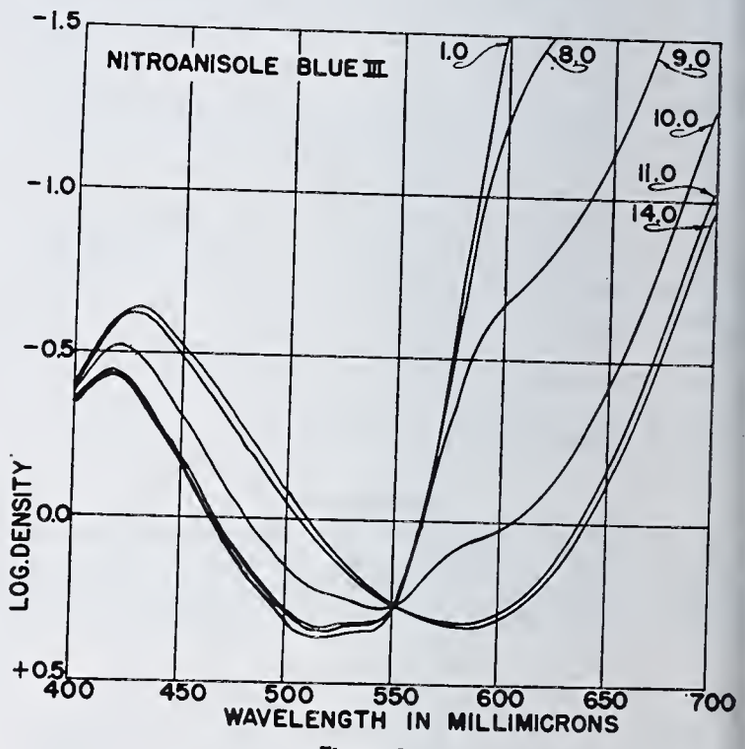

Figure 3

Epsilon blue (II) is noteworthy for its unusually high pK value, which recommends it for use in controlling alkalinity of di- and trisodium phosphate mixtures.

The solution colors described in Table II were made purposely weak in order to observe color changes under somewhat limiting conditions. Stronger solutions (0.2 to 0.3 gram per 100 ml.) are preferred in practice.

ACKNOWLEDGMENT

Thanks are due to J. H. McLeod and R. B. Payne for suggestions and preparation of the dyes.

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Theory and Operation of a Cartesian Diver Type of Manostat

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THE idea of using a float, consisting of an inverted tube with entrapped air, for vastly increasing the sensitivity of pressure measurements dates as far back as 1704, when Caswell published the details of a baroscope embodying this principle (*1*), which is generally known today as the Cartesian diver. More recently, the idea was modified to yield the low-pressure Dubrovin gage (*2*), the theory of which is described in a recent paper (*3*). An adaptation of the Cartesian diver to control the pressure in a closed system has been available for purchase for the past few years from the Emil Greiner Co., New York, N. Y., where the manostat was developed. This device has proved useful in adequately maintaining constant pressures in vapor-liquid equilibrium measurements and vacuum distillations ranging from a moderate vacuum of a few millimeters of mercury to atmospheric pressure.

OPERATION

Mercury is introduced into the container of Figure 1 until the disk of the float just makes contact with the orifice, when the pressure is equalized inside and outside the float. The device is connected to the vacuum pump, and to the system by way of a large reservoir and a suitable manometer. With the stopcock open, the pressure in the system is reduced by way of a by-pass between the pump and system until the desired value as read on the manometer is reached, then both the stopcock and by-pass are closed; the device will automatically maintain the desired pressure within limits determined by the dimensions of the constituent parts of the device. If the system is vacuum-tight, the pressure will maintain itself; however, a slight leak, which may be introduced intentionally, will cause the pressure to rise slightly. This will produce a displacement of the mercury level downward outside the float and a corresponding displacement upward inside the float; the buoyant force on the float is consequently diminished and when the reduction in buoyancy becomes sufficient to overcome the suction force at the orifice due to the pressure differential, the disk will break away from the orifice and permit the pump to evacuate sufficient gas from the system to restore the original pressure. When the original pressure is restored, the disk will return to its former position and seal off the orifice. The cycle is repeated indefinitely, if the size of the leak in the system does not exceed the capacity of gas flow that is possible through the orifice, and the pump is of sufficient rating to carry the load.

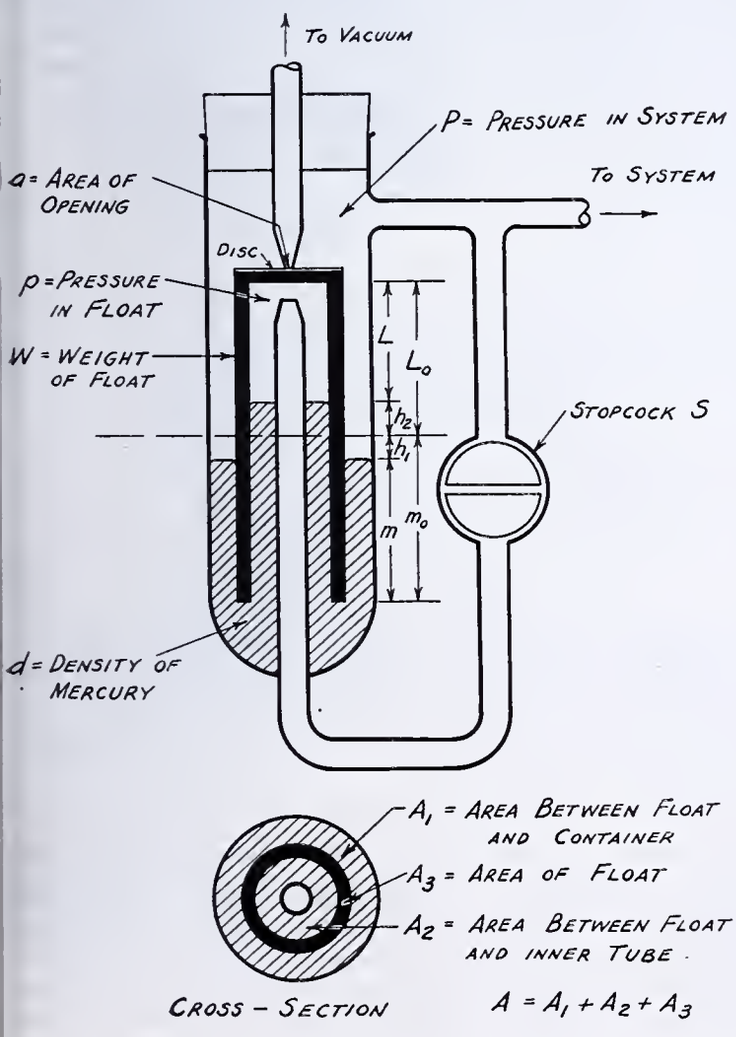


Figure 1

THEORY

In designing a Cartesian diver manostat, it is desirable to know the relationship between its sensitivity and physical dimensions, so that the particular requirements for its use may be met. Such a relationship is readily derived using the symbols of Figure 1.

By means of the stopcock, S, and the by-pass, the system is brought to the desirable pressure, P_0, as described above. The initial pressure inside the float, p_0, will be equal to that initially in the system, P_0, and the level of mercury inside and outside the float will be the same—i.e., h_1 and h_2 are initially 0. As the pressure increases in the system, owing to slight leaks, the liquid level is depressed outside the float and is correspondingly elevated inside the float, so that the buoyant force on the float is decreased. When the pressure in the system reaches a sufficiently high value, P, so that the buoyant force is diminished enough to counteract the force at the opening, a, due to the pressure differential, the float breaks away from the opening and the vacuum can restore the initial pressure. As soon as the initial pressure, P_0, is restored, the float cuts off the vacuum and the cycle is repeated indefinitely. The maximum variation in pressure, $P - P_0$, is a reciprocal measure of the sensitivity of the instrument and can be expressed quantitatively by setting up the following equations:

Pressure Equalities. $P = p + h_1 + h_2$ and $P_0 = p_0$ (pressure expressed as a height of mercury)

Combining, $$P - P_0 = p - p_0 + h_1 + h_2$$

and letting $$\Delta P = P - P_0 \text{ and } \Delta p = p - p_0$$

Therefore, $$\Delta P = \Delta p + h_1 + h_2 \qquad (1)$$

Equality of Volumes of Displaced Mercury.

$$h_1 A_1 = h_2 A_2 \qquad (2)$$

Isothermal Compression of Ideal Gas in Float.

$$pL = p_0 L_0$$

or $$pL - p_0 L = p_0 L_0 - p_0 L = p_0(L_0 - L) = p_0 h_2$$

and $L\,\Delta p = (L_0 - h_2)\,\Delta p = P_0 h_2$ (remembering that $P_0 = p_0$)

Therefore, $$L_0\,\Delta p = h_2(\Delta p + P_0) \qquad (3)$$

Equilibrium of Buoyant Forces. Assuming pressure in vacuum line is zero and neglecting buoyant forces due to gas,

$W + A_2(m_0 + h_2)d = (A_2 + A_3)md + aPd$, and $W = A_3 m_0 d$

Combining, $A_3 m_0 d + A_2 m_0 d + A_2 h_2 d = A_2 md + A_3 md + aPd$

Simplifying, $$h_1(A_2 + A_3) + h_2 A_2 = aP$$

Substituting Equation 2, and making use of $\Delta P = P - P_0$

$$h_1 A = a(\Delta P + P_0) \qquad (4)$$

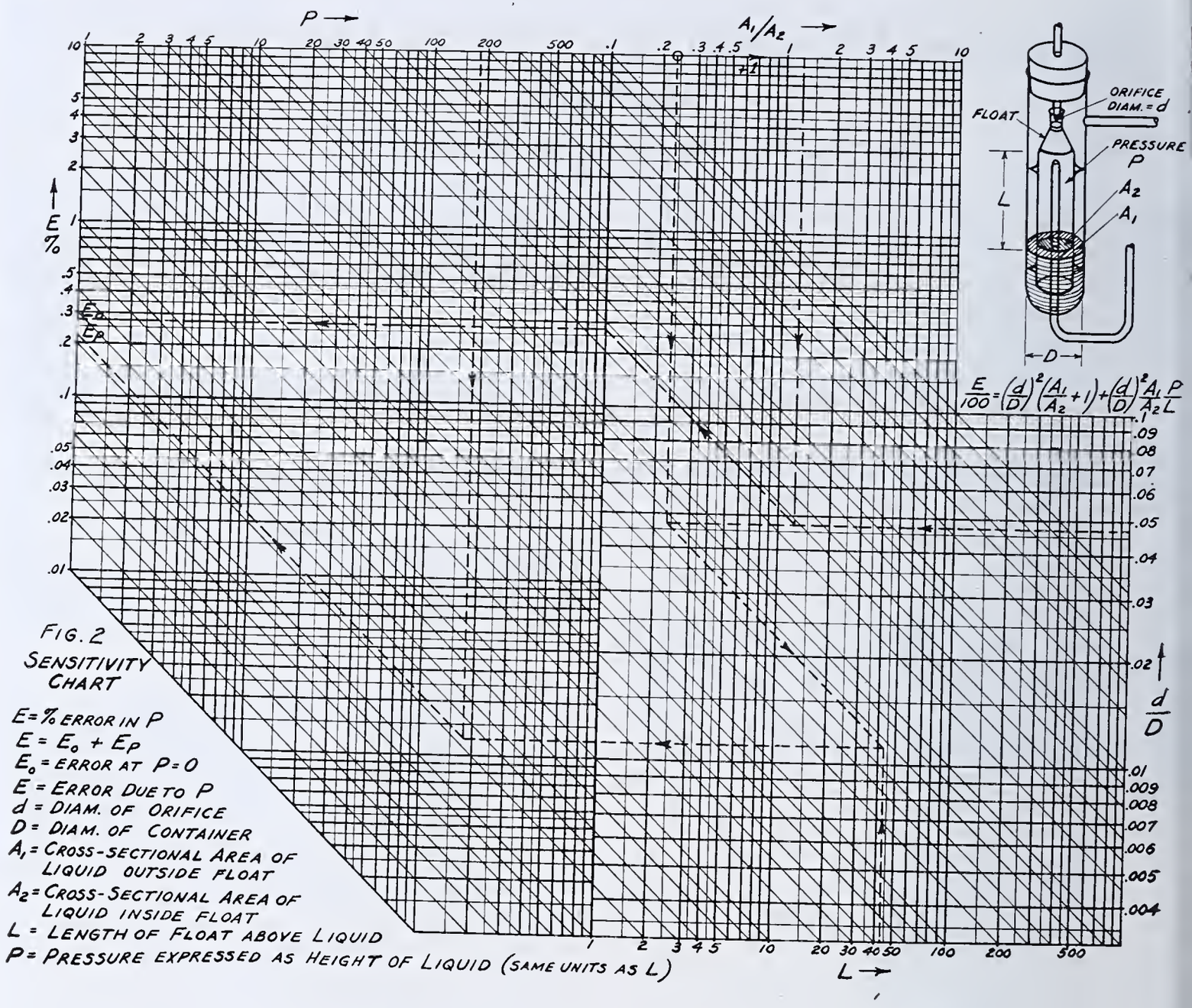


FIG. 2
SENSITIVITY CHART

Equations 1 to 4 may be solved simultaneously for ΔP in terms of P_0, L_0, a, A, A_1, and A_2 by eliminating h_1, h_2, and Δp:

Combining 2 and 4 to eliminate h_1 and letting

$$k_1 = \frac{a}{A}\,\frac{A_1}{A_2}$$

gives
$$h_2 = k_1(\Delta P + P_0) \tag{5}$$

Combining 3 and 5 to eliminate h_2 and simplifying,

$$\Delta p(L_0 - k_1\Delta P - k_1P_0) = k_1P_0(\Delta P + P_0) \tag{6}$$

Combining 1, 2, and 4 so as to eliminate h_1 and h_2 and letting

$$k_2 = \frac{a}{A}\left(\frac{A_1}{A_2} + 1\right)$$

gives
$$\Delta P(1 - k_2) - k_2P_0 = \Delta p \tag{7}$$

Combining 6 and 7 to eliminate Δp and simplifying,

$$(\Delta P)^2 - \left(\frac{L_0}{k_1} - 2P_0\right)\Delta P + \frac{k_2P_0L_0}{k_1(1 - k_2)} + P_0^2 = 0$$

Now k_1 and k_2 should be made very small to give high sensitivity, so that the square term may be neglected and $(1 - k_2)$ may be taken as unity; therefore as a close approximation,

$$\frac{\Delta P}{P_0} = \frac{k_2L_0 + k_1P_0}{L_0 - 2k_1P_0} = \frac{k_2 + \frac{k_1}{L_0}P_0}{1 - 2k_1\frac{P_0}{L_0}}.$$

As a further approximation, if k_1 is sufficiently small,

$$\frac{\Delta P}{P_0} = k_2 + \frac{k_1}{L_0}P_0 \tag{8}$$

Equation 8 expresses the sensitivity as a fractional deviation which is a linear function of the pressure when the above approximations are valid. From the definitions of k_1 and k_2, it is obvious that high sensitivity is obtained by making ratio $\frac{a}{A}$ as small as possible—i.e., by using a small opening and a large container. The sensitivity is also improved by making ratio $\frac{A_1}{A_2}$ smaller or L_0 larger. It is significant to note that the sensitivity is independent of the cross-sectional area of the float, A_3. Since the fractional deviation in the pressure to be maintained increases with the pressure, it is desirable to use a smaller opening, a, for larger pressures. Fortunately, this is in the desirable direction, because at higher pressures leakage is smaller and a higher pressure differ-

ential exists across the opening, so that more rapid evacuation is possible. To maintain very low pressures, a larger opening is required to take care of leaks and to give more rapid evacuation; since the last term in Equation 8 becomes negligible, the fractional deviation may then be conveniently approximated by:

$$\frac{\Delta P}{P_0} = k_2 = \frac{a}{A}\left(\frac{A_1}{A_2} + 1\right) \qquad (9)$$

To enable one rapidly to compute the sensitivity as given by Equation 8, the chart in Figure 2 was constructed. In this chart the symbols have the same meaning as before with the following addition:

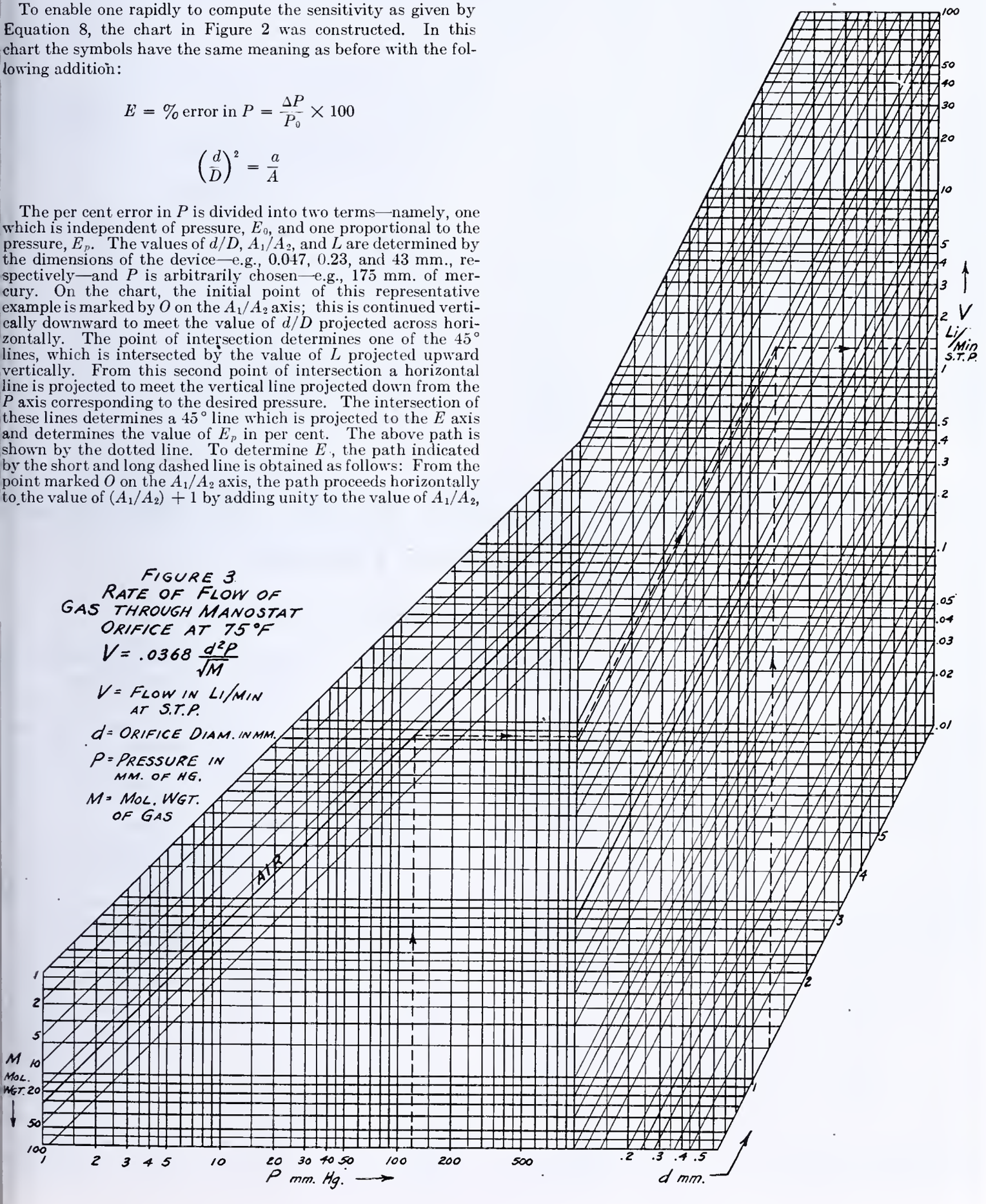
$$E = \%\text{ error in } P = \frac{\Delta P}{P_0} \times 100$$

$$\left(\frac{d}{D}\right)^2 = \frac{a}{A}$$

The per cent error in P is divided into two terms—namely, one which is independent of pressure, E_0, and one proportional to the pressure, E_p. The values of d/D, A_1/A_2, and L are determined by the dimensions of the device—e.g., 0.047, 0.23, and 43 mm., respectively—and P is arbitrarily chosen—e.g., 175 mm. of mercury. On the chart, the initial point of this representative example is marked by O on the A_1/A_2 axis; this is continued vertically downward to meet the value of d/D projected across horizontally. The point of intersection determines one of the 45° lines, which is intersected by the value of L projected upward vertically. From this second point of intersection a horizontal line is projected to meet the vertical line projected down from the P axis corresponding to the desired pressure. The intersection of these lines determines a 45° line which is projected to the E axis and determines the value of E_p in per cent. The above path is shown by the dotted line. To determine E, the path indicated by the short and long dashed line is obtained as follows: From the point marked O on the A_1/A_2 axis, the path proceeds horizontally to the value of $(A_1/A_2) + 1$ by adding unity to the value of A_1/A_2, and then vertically to the value of d/D, then at 45° to the axis corresponding to $L = 1$, and finally horizontally to give the value of E_0. The total per cent error in the pressure is the sum of E_0 and E_p:

$$E = E_0 + E_p = 0.28 + 0.22 = 0.50\%$$

Hence the pressure of 175 mm. of mercury will then fluctuate



by 0.9 mm. of mercury. Higher sensitivity is possible by decreasing the ratio d/D, say, by decreasing the size of the orifice. However, the size of the orifice limits the capacity of gas removal from the system, so that increased sensitivity by decreasing the size of the orifice is obtained at the expense of decreased capacity.

In Figure 3, the rate of gas flow is plotted from a suitable orifice flow equation (Fliegner's equation) modified to include the molecular weight of the gas, although otherwise based on air flow.

A representative example is shown by the dotted lines on the chart for:

$$P = 120 \text{ mm. of mercury, } d = 1.25 \text{ mm.}$$

$$\text{for air flow } (M = 29)$$

Starting at the value of P, the path proceeds vertically to the 45° line corresponding to the molecular weight of the gas (air is shown by the heavy line), then horizontally to the $d = 0.1$-mm. axis. From here the path follows an oblique line until it intersects the vertical line corresponding to the value of d, and finally horizontally to give the rate of gas flow of 1.3 liters per minute at standard temperature and pressure (S.T.P. = 0° C. and 760 mm. of mercury pressure).

Thus, by means of these charts, the dimensions of the device may be chosen to give desired characteristics within very wide ranges. These charts do not extend below a pressure of 1 mm. of mercury, because for lower pressures new factors controlling both the sensitivity and rate of gas flow are introduced. Thus, the rate of gas flow equation has to be modified by lower discharge orifice coefficients due to change-over from turbulent to viscous flow. Moreover, the sensitivity of the device becomes dependent upon the relative motion of the float, which is adequate at pressures above about 1 mm. of mercury; below this pressure, modifications in design are needed to magnify the displacement of the float.

CONCLUSIONS

The simplicity of this type of manostat and the ease with which it may be designed to meet a wide range of requirements make it ideal for practically all laboratory needs in place of complicated electrical hookups. Built of heavy glass or metal, the unit may be easily adapted for superatmospheric pressures. Furthermore, by suitable design, the device may be used for large industrial applications whenever constant-pressure conditions must be maintained automatically. Certain modifications of the device may also be made, so that it is more suitable for very low pressure, and in which it is also a direct-reading semivacuum gage. These modifications are still in the process of development and will be discussed in a subsequent paper.

ACKNOWLEDGMENTS

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Determination of Some Aromatic Amines and Substituted Ureas in Smokeless Powder

Improved Volumetric Bromination Procedure

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The volumetric bromination procedure which is in general use for the estimation of stabilizers in smokeless powder has been modified by the use of glacial acetic acid as a solvent for the sample to be brominated. The modified procedure is more convenient than the standard procedure in which carbon tetrachloride is used in a two-phase system, and less dependent on conditions of bromination than the standard procedure in which alcohol is used as the solvent.

FOR the quantitative determination of stabilizers in smokeless powders a volumetric bromination procedure has been widely used in preference to gravimetric procedures, which are generally more time-consuming. The volumetric bromination procedure involves treatment of a solution of a powder extract with a known amount of standard bromate-bromide solution, acidification to liberate bromine, addition of iodide in excess at the end of the bromination period, and titration of the free iodine with standard thiosulfate solution with the use of starch as indicator. This procedure has been used in the determination of diphenylamine, ethyl centralite (N,N'-diethyl-N,N'-diphenylurea), and acardite (N,N-diphenylurea). Under the conditions of the procedure 1 molecule of diphenylamine reacts with 4 molecules of bromine, and 1 molecule of ethyl centralite or acardite reacts with 2.

In the procedure as developed for centralite by Levenson (*2*), the bromination and titration are carried out in a one-phase solution in the presence of ethyl alcohol to keep the stabilizer in solution, under carefully controlled conditions of temperature and time in order to obtain quantitative bromination of the stabilizer without accompanying side reactions between the bromine and the alcohol. In order to eliminate the necessity for such precise control a procedure employing carbon tetrachloride as a solvent for the stabilizer was developed by Ellington and Beard (*1*). Carbon tetrachloride is nonreactive to bromine, so that conditions of bromination are much less critical than in the alcohol procedure. The manipulations are somewhat cumbersome, however, because the two-phase system must be shaken frequently during bromination and titration. Both the alcohol procedure and the carbon tetrachloride procedure are in common use for the estimation of diphenylamine and centralite in smokeless powders.

The authors have found that the advantages of both procedures may be obtained in a procedure in which glacial acetic acid is used as the solvent for the substances to be brominated. Acetic acid is nonreactive to bromine; moreover, its use permits the bromination and titration to be carried out in a single phase. Accordingly, they have developed a procedure involving the use of acetic acid and have tested this procedure by determinations of ethyl centralite, diphenylamine, and acardite.

Most procedures for the estimation of stabilizers in smokeless powder involve extracting the powder sample directly with ether or else decomposing the sample with alkali, distilling the stabilizer with steam, and extracting the distillate with ether; the re-

Table I. Analysis of Pure Samples of Stabilizers

Stabilizer	Method	Time of Bromination, Minutes	Temperature of Bromination, ° C.	Per Cent of Sample Detected: Individual determinations	Average
Ethyl centralite	HOAc	5	Room	101.5, 101.0, 102.0, 102.8	101.8
		1		100.2, 99.9, 99.8, 100.0	100.0
		45 sec.		99.8	99.8
		30 sec.		99.5	99.5
		15 sec.		99.3	99.3
	Alcohol	45 sec.	15–20	99.3, 99.6, 99.7, 99.2	99.5
	CCl_4	5	Room	99.8, 99.8, 98.3, 100.7, 99.9, 99.6, 100.8, 99.3, 99.6	99.8
Diphenylamine	HOAc	1	Room	99.9, 99.0	99.4
	CCl_4	5	Room	99.6, 99.8, 99.2, 99.9	99.6
Acardite	HOAc	10	Room	99.0	99.0
		5		99.1, 98.3	98.7
		1		84.5, 82.8	83.6
	CCl_4	3 hours	Room	81.3	81.3
		5		30.9	30.9

Table II. Comparative Analyses of a Nitroglycerin Propellant for Ethyl Centralite

	Carbon Tetrachloride %	Acetic Acid %
Centralite	0.986	0.971
	0.997	0.973
		0.978
		0.981
Av.	0.992	0.976

ulting ether solution is evaporated, and the residue from the evap-ration is analyzed for stabilizer. The authors have found that he use of these procedures on samples that were subsequently nalyzed for ethyl centralite or diphenylamine by the carbon tet-achloride bromination procedure frequently led to results which ere low by as much as 10% of the stabilizer present. Subsequent tudies indicated that the discrepancies were due to the presence f difficultly volatile peroxides in the ether. The replacement of ther by methylene chloride as an extracting solvent eliminated he discrepancy entirely and led to satisfactory results. Accord-ngly its use is recommended in the procedure given below.

PROCEDURE

This procedure may be used when diphenylamine, centralite, or cardite are present in a smokeless powder in the absence of other ubstances that react with bromine. Nitroglycerin and diethyl hthalate do not interfere. When more than one stabilizer is pres-nt in a given powder, a more involved procedure than the one iven below must be used.

Reagents. Standard 0.1000 N bromate-bromide solution is repared by dissolving 2.784 grams of recrystallized potassium romate (dried to constant weight at 110° C.) and 15 grams of otassium bromide in distilled water and diluting to 1 liter in a olumetric flask. Standard 0.05 N sodium thiosulfate solution, .5% starch indicator solution, and a 15% solution of potassium odide are prepared according to standard iodometric practice 3).

The methylene chloride used for extraction is distilled from echnical grade material; adequate head and tail fractions are dis-arded. Reagent grade glacial acetic acid is used in the bromina-ion procedure.

Extraction and Bromination. A sample of powder con-aining not more than 0.075 gram of ethyl centralite, 0.02 gram of iphenylamine, or 0.06 gram of acardite is finely divided and in-roduced into a Soxhlet extraction apparatus, which is attached to 250-ml. glass-stoppered iodination flask containing 100 ml. of nethylene chloride. The sample is extracted for 2 hours or more, epending on its state of subdivision. The flask is then detached nd the methylene chloride is completely evaporated by means of stream of dry air, with suitable precautions to minimize the azard due to possible detonation of the nitroglycerin in the ex-ract. The residue is dissolved by the addition of 60 ml. of glacial cetic acid, and 25.00 ml. of 0.1000 N bromate-bromide solution are dded with a pipet. Five milliliters of concentrated hydrochloric cid are added, the flask is stoppered, and the contents are mixed y swirling. The bromination is allowed to proceed for 1 ± 0.25 inutes from the time the solution was acidified if centralite or iphenylamine are being determined, or at least 5 minutes in the ase of acardite.

Titration. At the end of this time 10 ml. of 15% potassium iodide solution are added, the flask is swirled, and the gutter and walls are washed down with distilled water from a wash bottle. The solution is titrated immediately with 0.05 N sodium thiosulfate solution. Five milliliters of 0.5% starch solution are added when the solution has assumed a light yellow color, and the titration is continued to the disappearance of the blue color. A blank determination is made, under the same conditions of bromination and titration, on a 60-ml. portion of glacial acetic acid. The percentage of stabilizer in the powder sample is calculated by means of the equation

$$\text{Percentage of stabilizer} = \frac{(1 - A/B)\,NVC}{W}$$

in which A = volume of thiosulfate consumed in titration of sample
B = volume in milliliters of thiosulfate solution consumed in titration of blank
N = normality of standard bromate-bromide solution
V = volume of pipet in milliliters
W = weight of powder sample in grams
C = one tenth of equivalent weight of stabilizer; 6.709 for centralite, 2.115 for diphenylamine, and 5.306 for acardite

The nitroglycerin remaining in the solution at the end of the titration should be destroyed by boiling with an excess of ferrous chloride or by some other appropriate procedure.

RESULTS

The accuracy and precision of the new method and the effect of time of bromination were determined by analysis of purified samples of ethyl centralite, acardite, and diphenylamine, as well as powder samples containing ethyl centralite; comparison analyses were also made by one or both of the standard procedures mentioned above. The results of these experiments are presented in Tables I and II. Additional experiments, the results of which are not given in the tables, demonstrated that acetic acid is virtually inert toward bromination under conditions of the procedure and that the presence of either nitroglycerin or diethyl phthalate does not introduce serious error into the estimation of centralite.

The data in Tables I and II demonstrate that this volumetric procedure involving bromination in acetic acid solution is satisfactorily accurate and precise, and that the results are not critically dependent on the time allowed for bromination. As it is more convenient to carry out than the carbon tetrachloride and alcohol procedures, it is recommended for the estimation of stabilizers in smokeless powders.

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Analysis of Organoselenium Compounds

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A procedure developed for the determination of selenium in organic compounds involves combustion of the substance in a hydrogen-oxygen flame, followed by collection and titration of the selenium dioxide formed. Iodometric methods for the determination of the equivalent weights of certain types of organoselenium compounds reduced by potassium iodide and of diaryl diselenides are also described. The methods are all rapid and accurate.

INVESTIGATION of the known methods for the analysis of organic compounds of selenium showed these procedures to be either tedious and time-consuming or inaccurate. In testing the available methods, best results were obtained by use of a modification of the combustion method of Niederl and Niederl (*4*) which is based on the methods of Alber and Harand (*1*) and Umezawa (*5*) and involves combustion of the substance in oxygen gas on a glowing platinum surface. However, it was found more expedient and fully as accurate to determine the selenium dioxide formed by an improved iodometric method based on that of van der Meulen (*3*). The titration as described by van der Meulen is erratic, particularly where hydrochloric acid is used or where chlorine is present in the substance being analyzed. This erratic behavior is due to oxidation of iodide by oxygen of the air which is apparently catalyzed by something present in the solution. This difficulty was overcome by modifying the method as described in the procedure below. In spite of this and other improvements, the method was still lengthy and required constant attention and skill during the combustion. It gives good results, however, in the hands of a skilled operator.

The new analytical methods described in the present paper are of two types: (1) the flame combustion of the organoselenium compound which leads directly to the determination of the selenium content and (2) volumetric methods which yield the equivalent weights of the compounds being analyzed.

FLAME COMBUSTION METHOD

A method for the determination of halogens in organic compounds by flame combustion has been described by Winter (*6*). The simplicity and accuracy of this procedure led the present workers to investigate its possibilities as a method for selenium analysis. Preliminary tests showed that selenium was oxidized completely to the tetravalent state when the organoselenium compound was burned in a flame supplied with an ample quantity of oxygen. In an ordinary air-supplied gas flame, part of the selenium came through as the element and formed a red deposit in the chimney.

APPARATUS AND PROCEDURE. The apparatus is shown in Figure 1. The burner, *ABCD*, is made of transparent quartz tubing. Although the dimensions do not seem to be critical, the inside diameters of the tubing actually used are 6 mm. for arm *AB*, 3 mm. for side arm *D* and the inner jet of burner *C*, and 10 mm. for the outer tube of the burner. The chimney and absorption tube, *EFGH*, are of Pyrex, the inside diameters being 30 mm. at *E*, 6 mm. at *F*, and 15 mm. at *H* with a 10/30 ⅀ joint at *G*. Other dimensions are shown roughly to scale in the figure. The absorption tube, *H*, is tightly packed with Pyrex glass-wool which had been soaked in chromic acid cleaning solution and thoroughly rinsed with water.

In use, the burner, thermometer, and lower part of the chimney are placed inside an electric furnace, *L*, contained in a 10 × 20 cm. Pyrex electrolytic beaker, *K*. The heater consists of 5.4 meters (18 feet) of 22-gage Chromel-A wire (1.0 ohm per foot) wound on a Transite frame and is energized through a variable transformer.

The apparatus is assembled into three independently movable units consisting of the quartz burner and thermometer, the furnace, and the chimney and absorption apparatus.

Although only solid samples have been analyzed in this labora tory, there is no apparent reason why liquid and gaseous sample could not be handled if one modified the apparatus as describe by Winter for halogen analysis. The sample size was adjuste to contain 20 to 80 mg. of selenium, the governing factors be ing the normality of the sodium thiosulfate, the size of the buret and whether or not a method of aliquots is employed in th titration. The sample may be compressed into a pellet o weighed directly into the small platinum-foil boat, *B*.

The weighed sample is introduced at *A* and shaken down to *B* Hydrogen gas is then introduced at *D* at a rate of about 200 ml per minute and after the original air has been swept out, the ga is lit at *C*. The flame should be about 1 cm. high. The oxyge is then slowly turned on until a flow rate of about 100 ml. pe minute is reached. Once started, the flame is very stable an does not go out during an analysis. The furnace and chimne assemblies are then brought into place and suction applied at sufficient to give an air flow through the chimney of about liter per minute, which is rapid enough to sweep all products o combustion into the absorber. Although successful analyse have been performed without the use of flowmeters, it is recom mended that some kind of gage be placed in each of the three line mentioned.

The current to the heater is then started and the voltage raise to the point where a suitable rate of volatilization of the sample i obtained. Experience alone will tell the most satisfactory tem perature for the vaporization of a given substance, but the opera tor soon becomes skilled in this regard. The otherwise color less flame assumes a blue color, the intensity of which is propor tional to the rate at which the selenium compound is being burned This color serves as a good indication of the onset of vaporizatio as well as of the completion of the analysis. Most of the seleniu dioxide formed in the reaction deposits in the tube at *F*, but a significant quantity makes its way to the glass-wool in the ab sorber, *H*.

A large proportion of the compounds analyzed vaporized cleanly without decomposition or residue. However, in some cases decomposition accompanies volatilization, so that a dark, nonvolatile, selenium-containing residue remains. When this is the case, the thermometer is removed and the temperature is gradually raised to the point where the compound slowly burns in the oxygen stream. This is the advantage of introducing the sample into the oxygen rather than into the hydrogen. In some cases the decomposition can be prevented by vaporizing at a higher temperature, the rate of vaporization being increased more than the rate of decomposition as the temperature rises. Increasing the rate of oxygen flow also helps in these cases.

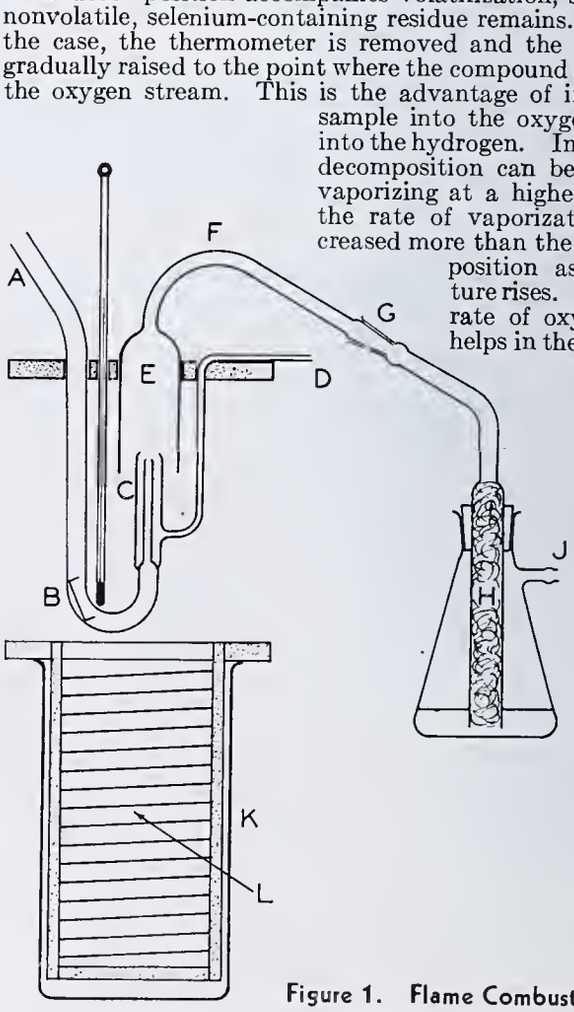

Figure 1. Flame Combustion Apparatus

Table I. Analysis of Organoselenium Compounds by the Flame Combustion Method

Compound	Formula	Selenium Theory %	Selenium Analysis %	Deviation %
Di-*p*-tolylselenium	$C_{14}H_{14}Se$	30.23	30.22	−0.03
Dibenzyl diselenide	$C_{14}H_{14}Se_2$	46.42	46.53	0.24
Diphenyl diselenide	$C_{12}H_{10}Se_2$	50.60	50.51	−0.18
Bis (*p* - methoxyphenyl) selenium	$C_{14}H_{14}O_2Se$	26.93	26.83	−0.37
Bis (*o* - chlorophenyl) selenium	$C_{12}H_8Cl_2Se$	26.14	26.14	0.00
p-Tolyl selenocyanate	C_8H_7NSe	40.27	40.37	0.25
p - Nitrophenyl selenocyanate	$C_7H_4N_2O_2Se$	34.77	34.60	−0.49
Phenylseleninic acid	$C_6H_6O_2Se$	41.76	41.70	−0.14
α - (*o* - Biphenylylseleno) propionic acid	$C_{15}H_{14}O_2Se$	25.87	25.77	−0.39

When combustion is complete, which requires 15 to 45 minutes, the hydrogen and oxygen are stopped and the furnace is shut off and lowered to permit the apparatus to cool. The suction is left on during the cooling process. When cool, the chimney is removed from the absorber and the selenium dioxide rinsed into a 250- or 500-ml. volumetric flask with water. By alternately applying suction to *G* and *J*, the glass-wool is rinsed with three or four 25-ml. portions of water, each portion being drawn up and down slowly several times, then added to the volumetric flask. This rinsing must be carefully done in order to remove all the selenious acid. The final rinse water should not show a color when added to an acidified solution of starch and potassium iodide. After rinsing is complete, the volumetric flask is filled to the mark and aliquots are titrated by the procedure below.

Titration of Selenious Acid. The solution containing the selenious acid is boiled gently for a few minutes to remove dissolved oxygen, then cooled to room temperature in an ice-water mixture. Air is subsequently kept out of the flask by addition of small pieces of dry ice from time to time or by passage of carbon dioxide or nitrogen gas into the flask. Although this latter precaution is not absolutely necessary, more reliable results have been obtained in this way. To the solution are then added 15 ml. of 2% starch solution, 10 ml. of 1.5 *M* potassium iodide, and 10 ml. of 6 *N* sulfuric acid in the order named, mixing thoroughly after each addition. The solution is then titrated at once with approximately 0.05 *N* sodium thiosulfate. The end point is sharp and is marked by a change from a turbid brown to a transparent red color. 1.000 ml. of 0.05000 *N* $Na_2S_2O_3$ = 0.9870 mg. of Se.

Results and Discussion. The accuracy of the flame combustion method is indicated by the results given in Table I for the analysis of a number of carefully purified substances.

The above examples show that the method yields excellent results for compounds of widely differing types containing selenium together with carbon, hydrogen, oxygen, nitrogen, and chlorine. The method in its present form does not give good results when bromine, iodine, or sulfur is present, since these elements yield substances which reduce tetravalent selenium in the absorber. However, bromine and iodine also interfere with other combustion methods, although sulfur does not because of its oxidation to sulfur trioxide (rather than dioxide) on the platinum contacts. It is this interference by sulfur in the flame combustion method which makes it advisable to use hydrogen as a fuel rather than commercial gas which is apt to contain sulfur. The flame combustion method has a distinct advantage over other methods in being more rapid and requiring less skill and attention on the part of the analyst.

VOLUMETRIC METHODS

A number of types of organoselenium compounds which contain halogen or oxygen directly bonded to selenium lend themselves to iodometric procedures for determination of equivalent weight. These compounds react with aqueous potassium iodide to form triiodide, which may then be titrated with sodium thiosulfate. The titration of diarylselenium dihalides has been described in a previous communication (*2*). The reduction products and the equivalents per mole for the various types of compounds are indicated in Table II.

Table II. Reduction Products and Equivalents per Mole for Various Types of Organoselenium Compounds

Type of Compound	Reduction Product	Equivalents per Mole
R_2SeX_2	R_2Se	2
R_2SeO	R_2Se	2
$RSeX_3$	R_2Se_2	3
$RSeX$	R_2Se_2	1
$RSeO_2H$	R_2Se_2	3

Table III. Equivalent Weights of Some Selenium Compounds Reduced by Potassium Iodide

Compound	Formula	Equivalent Weight Theory	Equivalent Weight Experimental	Deviation, %
Diphenylselenium dichloride	$(C_6H_5)_2SeCl_2$	152.1	151.9	−0.13
Diphenylselenium dibromide	$(C_6H_5)_2SeBr_2$	196.5	196.6	0.05
Di-*p*-tolylselenium dibromide	$(C_7H_7)_2SeBr_2$	210.5	210.3	−0.10
Dibenzoselenophene dibromide	$C_{12}H_8SeBr_2$	195.5	195.4	−0.05
4 - Methoxydiphenylselenium dichloride	$C_{13}H_{12}OSeCl_2$	167.1	167.7	0.36
4 - Bromodiphenylselenium dibromide	$C_{12}H_9BrSeBr_2$	236.0	235.1	−0.38
4 - Methyldiphenylselenium dibromide	$C_{13}H_{12}SeBr_2$	203.5	203.6	0.05
4,4′ - Diethoxydiphenyl selenoxide	$(C_8H_9O)_2SeO$	168.6	169.0	0.24
Phenylselenium tribromide	$C_6H_5SeBr_3$	131.9	131.4	−0.30
Phenylselenium monobromide	C_6H_5SeBr	236.0	236.3	0.13
Phenylseleninic acid	$C_6H_6SeO_2H$	63.0	63.2	0.32

Table IV. Iodometric Titration of Diaryl Diselenides

Compound	Formula	Equivalent Weight Theory	Equivalent Weight Experimental	Deviation, %
Diphenyl diselenide	$(C_6H_5)_2Se_2$	52.01	51.97	−0.1
			51.53	−0.9
Di-*p*-tolyl diselenide	$(C_7H_7)_2Se_2$	56.70	56.98	0.5
Di(*o*-biphenylyl) diselenide	$(C_{12}H_9)_2Se_2$	77.38	76.84	−0.7

Procedure for Compounds Reducible by Potassium Iodide. The weighed sample (0.1 to 0.5 gram) is placed in a glass-stoppered flask containing 5 ml. of carbon tetrachloride, 25 ml. of 0.3 *M* potassium iodide, and 2 ml. of 6 *M* sulfuric acid. After shaking, the mixture is titrated with standard sodium thiosulfate. Starch solution is added just before the end point.

Titration of Aryl Diselenides. Aryl diselenides may be titrated iodometrically to the iodine monochloride end point if the hydrochloric acid concentration is kept at 5.0 to 5.5. *F* at the end point. Because of a small amount of side reaction, possibly oxidation of the diselenide to selenonic acid, the results are sometimes low by 0.5 to 1.0%. Although the reactions are probably more complex than indicated, the stoichiometry is represented by the equations:

$$R_2Se_2 + 6\ ICl = 2\ RSeCl_3 + 3\ I_2 \quad (1)$$

$$2\ I_2 + IO_3^- + 6H^+ + 5\ Cl^- = 3\ H_2O + 5\ ICl \quad (2)$$

Procedure for Aryl Diselenides. A solution of iodine monochloride is prepared by titration of 1 ml. of 1.5 *M* potassium iodide in 50 ml. of 12 *M* hydrochloric acid, using 5 ml. of carbon tetrachloride as an indicator. When the carbon tetrachloride is just colorless, 25 ml. of 12 *N* hydrochloric acid and the weighed sample of diselenide are added. After shaking, the mixture is titrated with standard potassium iodate solution.

Tables III and IV show results from volumetric determination of equivalent weights of a number of different compounds.

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Oxide Films Formed on Alloys at Moderate Temperatures

Electron Diffraction and Electron Microscope Study

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Supplementing previous papers, electron microscope and electron diffraction data are presented concerning the structure of electrochemically and chemically stripped films from a series of 16 alloys consisting principally of iron, cobalt, nickel, and chromium. Both reflection and transmission methods of electron diffraction were used.

THE nature of the protective properties of metals and alloys is a question of great importance in modern technology. Many theories have been proposed, some of which are based on the role of the oxide film in preventing further reaction. A complete knowledge of the chemical and physical structure of the oxide crystals in the surface film may aid in determining the necessary conditions for protection, but unfortunately, a complete knowledge is impossible for very thin films with the present state of instrumentation. In previous papers (*8*, *10*, *15*) the use and limitations of the electron diffraction and electron microscope techniques were discussed. This paper presents electron microscope and electron diffraction data concerning the structure of electrochemically and chemically stripped films from a series of 16 alloys consisting principally of iron, cobalt, nickel, and chromium.

SURVEY OF LITERATURE

The literature on the removal of oxide films from iron and other metals by chemical and electrochemical methods developed largely by Evans and co-workers (*4*) was reviewed in a previous paper (*15*).

The electrochemical method has been applied to the stripping of oxide films from alloys of iron and chromium by Evans and Stockdale (*5*). A very thin film of oxide was stripped from 13% chromium steel by a long anodic treatment. The film, which carried striae of the original metal surface, also contained opaque flakes. These workers also obtained from 18-8 stainless steel a thin skin which contained large amounts of residual metal. The film was opaque to light.

The chemical method has been applied with success to the removal of the surface film on 18-8 stainless steel by Vernon, Wormwell, and Nurse (*17*). The metals present in the film were determined by chemical analyses and the contents of the corresponding oxides computed. The thickness of the oxide film increased with the degree of polish. The effect of polishing was to enrich the chromium in the film as compared with the underlying steel. The surface film of brightly polished specimens contained 90% chromic oxide. Nickel, on the other hand, was not found to be enriched in the surface film. The authors have suggested that the enrichment of chromium is associated with surface flow.

In the macroscopic film thickness range considerable information is available on the enrichment and concentration of the several metals making up the oxide film. Pfiel (*14*) has made chemical analyses on films of the order of 0.25 cm. (0.1 inch) or more in thickness. These films formed at temperatures of the order of 1000° C. existed in the form of three layers. In a study of eight alloys the two outer layers contained only very small amounts of the alloying elements compared with the original steel except in the case of manganese steel. With a few exceptions nonferrous elements present in the alloy before oxidation were concentrated in the innermost of the three layers of scale. Pfiel (*14*) considers the oxidation process as one involving iron atoms diffusing outward through the oxide film.

Recently Kornilov and Sidorishin (*11*) investigated the oxide films formed on iron-chromium-aluminum solid solution alloys by electron diffraction and chemical analysis in the temperature range 400° to 1000° C. At moderate temperatures an isomorphous mixture of oxides of the spinel type was found. With increasing temperature the lattice constant of the solid solution of the oxides decreased and approached that of pure γ-alumina. The oxide film which formed on the surface crumbled readily and had no protective properties.

Table I. Analysis and Preparation of Alloy Specimens

Alloy	Analysis	Heat Treatment, Hours at 1000° C., Furnace Cooled	Polishing
Mild steel[a]	0.18 C, 0.028 S, 0.030 P	15 in dry H_2	Emery papers through 2/0, uniwax wheel, 320 abrasive, uniwax wheel 600 aloxite, No. 3 alumina
13 CrFe	13 Cr	10 in dry H_2	Emery papers through 1/0, wax wheel, 320 abrasive, chrome rouge, Nos. 1 and 3 alumina
18-8 SS	18 Cr 8 Ni	15 in Ammogas	Emery papers through 2/0, chrome rouge, Nos. 1 and 3 alumina
K42B[a]	41.7 Ni, 22.5 Co, 20.0 Cr, 12.0 Fe, 1.89 Ti. 0.21 Al, 0.57 Mn, 1.21 Si	10 in wet H_2	Emery papers through 2/0, chrome rouge, Nos. 1 and 3 alumina
Inconel (*3*)	79.5 Ni, 13 Cr, 6.5 Fe, 0.25 Mn, 0.25 Si, 0.08 C, 0.20 Cu	15 in Ammogas	Emery papers through 1/0, 320 abrasive wheel, chrome rouge, Nos. 1 and 3 alumina
Nichrome V	80 Ni, 20 Cr	14 in Ammogas	Emery papers through 1/0, 320 abrasive wheel, chrome rouge, Nos. 1 and 3 alumina
30 CoFe[a]	30.4 Co, 0.22 C	10 in dry H_2	Emery papers through 3/0, chrome rouge, Nos. 1 and 3 alumina
Hipernik	49 Ni, 49 Fe, 2 Mn	10 in dry H_2	Emery papers through 1/0, wax wheel, 320 abrasive, chrome rouge, Nos. 1 and 3 alumina
5 CrFe[a]	4.63 Cr, 0.044 C	10 in dry H_2	Emery papers through 1/0, wax wheel, 320 abrasive, chrome rouge, Nos. 1 and 3 alumina
5 NiFe[a]	4.89 Ni, 0.015 C	10 in dry H_2	Emery papers through 1/0, wax wheel, 320 abrasive, chrome rouge, Nos. 1 and 3 alumina
5 CoFe	.....	10 in dry H_2	Emery papers through 3/0, chrome rouge, Nos. 1 and 3 alumina
5 MnFe[a]	4.85 Mn, 0.035 C	10 in dry H_2	Emery papers through 3/0, chrome rouge, Nos. 1 and 3 alumina
5 SiFe	.....	10 in dry H_2	Emery papers through 3/0, chrome rouge, Nos. 1 and 3 alumina
3 VFe	.....	10 in dry H_2	Emery papers through 3/0, chrome rouge, Nos. 1 and 3 alumina
Kovar	54 Fe, 18 Co, 28 Ni	10 in dry H_2	Emery papers through 3/0, chrome rouge, Nos. 1 and 3 alumina
4 WFe[a]	4.10 W, 0.028 C	10 in dry H_2	Emery papers through 3/0, chrome rouge, Nos. 1 and 3 alumina

[a] Analyzed at research laboratories.

APPARATUS AND METHOD

The details of the apparatus and methods used in this study have been described (*8*, *10*).

The specimens of the alloys are heat-treated and given a metallographic polish according to the procedures tabulated in Table I. The samples are then mounted in the electron diffraction camera furnace and oxidized under carefully controlled conditions of time, temperature, and oxygen pressure. The surface lattice structure is studied in situ by the electron diffraction reflection method. Three photographs are taken: (1) the sample in vacuum and at the temperature of the experiment; (2) after the oxidation and while the specimen is at the elevated temperature; (3) after the specimen is cooled under vacuum conditions to room temperature.

The sample is now removed and cut into two pieces. Light micrographs are made of the surface of one half of the specimen by reflected light at 100 and 1000×. The other half is subjected to the electrolytic or chemical stripping techniques (*15*). After the film is loosened, it is washed and then manipulated carefully onto a small stainless-steel specimen screen. The screen and specimen are vacuum-dried before being placed in the microscope for study. Several electron micrographs are taken at 6800× of typical portions of the stripped oxide film. The film is also studied by the electron diffraction transmission method, using the electron diffraction adapter of the electron microscope.

CHOICE AND PREPARATION OF SPECIMENS

The alloys in this study may be divided into four general classes, although a given alloy may belong to two or more classes—for example, both Inconel and K42B are refractory alloys but they also have good protective qualities. The following classification is used in the discussion of results:

1. Protective. 13 CrFe, 18-8 stainless steel
2. Refractory. K42B, Inconel, Nichrome V
3. Magnetic. Hipernik, 30 CoFe
4. Sealing. Kovar

In addition to commercial alloys, certain experimental alloys were made to determine whether the lattice type of the alloying element would have any effect on the oxidation products. These ferrous alloys contain alloying metals of the body-centered cubic, face-centered cubic, and other structural types. In each the percentage of the alloying metal does not exceed 5%.

The specimens are machined from bars of the alloys to cylinders of 0.94 cm. (0.375-inch) diameter and 0.94 cm. (0.375-inch) length. After cleaning, they are heat-treated at elevated temperatures in dissociated ammonia or wet or dry hydrogen. The specimens are next given a fine metallographic polish. Details of the heat-treatment and polishing procedures are given in Table I. The specimens are stored in a desiccator over anhydrous calcium chloride until used.

The alloy specimen is placed in the electron diffraction camera furnace and heated to the desired temperature. Oxygen to a pressure of 0.1 atmosphere is admitted and the specimen is oxidized for a predetermined time. The time and temperature conditions of the oxidation are estimated from rate measurements where these are available. For most of the alloys no rate measurements are available, so that intelligent guesses as to oxidation conditions are necessary in order that the oxide film thickness shall lie within the optimum range for investigation with the electron microscope.

INTERPRETATION OF DATA

Electron Diffraction. The electron diffraction reflection method has been discussed in previous papers (*8*, *10*, *15*) and the interpretation of data by the transmission technique has been discussed in a recent work on metals (*15*). The transmission method yields information on the structure of the whole film, while the reflection method may indicate only the structure of the outer surface. This is important, since the surface structure may not be the same as the structure of the body of the film. The oxide layer which forms initially may consist of various oxides present on the surface in the same mole ratio as the metals in the alloy. As the oxidation proceeds a stratification of layers of the several oxides may occur, since the factors influencing their formation may be different. Thus, in a binary alloy, an oxide of one of the metals may concentrate in the surface layer while an oxide of the other metal may concentrate in contact with the metallic substrate.

Table II. Lattice Parameters of Metals and Metallic Oxides

Substance	*a*	*b*	*c*	α	Structural Type
Fe	2.86	...	...	.....	Body-centered cubic
W	3.16	...	...	.....	Body-centered cubic
Cr	2.88	...	...	.....	Body-centered cubic
V	3.03	...	...	.....	Body-centered cubic
Ni	3.52	...	...	.....	Face-centered cubic
Co	2.51	...	4.07	.....	Hexagonal close-packed
Mn	8.89	...	...	.....	Cubic
Si	5.42	...	...	.....	Cubic (diamond)
FeO	4.28	...	...	.....	Face-centered cubic
CoO	4.25	...	...	.....	Face-centered cubic
NiO	4.17	...	...	.....	Face-centered cubic
WO_2	4.86	...	2.77	.....	Tetragonal
Cr_2O_3	5.35	...	...	54°58′	Rhombohedral
α-Fe_2O_3	5.42	...	...	55°17′	Rhombohedral
W_2O_3	...	...	...	.....	Rhombohedral
WO_3	7.28	7.48	3.82	.....	Monoclinic
V_2O_3	5.43	...	...	53°53′	Rhombohedral
V_2O_5	11.48	4.36	3.55	.....	Orthorhombic
SiO_2	4.90	...	5.39	.....	Hexagonal
MnO	4.44	...	...	.....	Face-centered cubic
MnO_2	4.44	...	2.89	.....	Tetragonal
Mn_3O_4	5.75	...	9.42	.....	Tetragonal
Fe_3O_4	8.40	...	...	.....	Spinel (cubic)
Co_3O_4	8.11	...	...	.....	Spinel (cubic)
γ-Fe_2O_3	8.32	...	...	.....	Cubic
$MnO.Cr_2O_3$	8.42	...	...	.....	Spinel (cubic)
$NiO.Cr_2O_3$	8.31	...	...	.....	Spinel (cubic)
$CoO.Cr_2O_3$	8.32	...	...	.....	Spinel (cubic)
$FeO.Cr_2O_3$	8.35	...	...	.....	Spinel (cubic)
$MnO.Fe_2O_3$	8.51	...	...	.....	Spinel (cubic)
$NiO.Fe_2O_3$	8.34	...	...	.....	Spinel (cubic)
$CoO.Fe_2O_3$	8.39	...	...	.....	Spinel (cubic)

The interpretation of the information obtained by the electron diffraction method is more difficult in the case of alloys than of metals. Because of the similarity of the lattice parameters of many of the oxides, there is difficulty in distinguishing α-Fe_2O_3 from Cr_2O_3, $NiO.Fe_2O_3$ from Fe_3O_4, etc. Table II shows the lattice parameters of the various oxides of interest in this study. Other complicating factors are the phenomena of various types of solid solution of the several oxides in each other. For example, on an iron-chromium alloy where both α-Fe_2O_3 and Cr_2O_3 may be present it is difficult by the electron diffraction technique to determine whether α-Fe_2O_3 or Cr_2O_3 is present or a solid solution of one in the other. There may also occur a random replacement of ferric ions by chromic ions in the Fe_3O_4 (spinel type) lattice. The occurrence of such solid solution phenomena may have effects on the lattice parameters and thus complicate the identification of the oxidation products.

An interesting comparison can be made of the reflection and transmission electron diffraction patterns. In general, one should expect to find certain oxidation products by reflection and additional products by transmission. However, the oxides in the outer layer as determined by reflection may comprise such a small fraction of the complete oxide film that one or more of them may not give good diffraction patterns in the transmission investigations.

Electron Microscope. The two methods of studying surfaces of opaque bodies by means of the electron microscope have been compared in a recent work on metals (*15*). Since we are interested primarily in the details of crystals making up the body of the oxide film, the stripped film technique is used exclusively in this study.

Information Recorded. Electron micrographs of the stripped oxide film are taken at 6800× and enlarged optically to 34,000×. The following information is recorded from the electron micrographs of the stripped film: (1) particle size, (2) particle size distribution, (3) particle shape, (4) uniformity in film thickness, and (5) type of micrograph. The particle size is obtained by averaging measurements on a number of crystals, while the particle size distribution indicates the variation in particle size. The particle shape is determined from an examination

Table III. Electron Diffraction Data

(Oxide films formed on alloys at 0.1 atmosphere of O_2 at various times and temperatures)

Alloy	Temp. °C.	Time of Oxidation Min.	Diffraction Patterns: Preoxidation by R	Oxidized by R	Oxidized at 25° C. by R	Stripped by T
Protective						
13 CrFe	600	5	None	Cr_2O_3* MO	Cr_2O_3, M O	Cr_2O_3, Fe_3O_4*, 8.41, S
18-8 SS	600	5	α-Fe_2O_3, D	Fe_2O_4* 8.44, α-Fe_2O_3, S	Fe_2O_4*, 8.44, α-Fe_2O_3, S	Cr_2O_3, S
18-8 SS	600	40	Fe_3O_4, 8.45, α-Fe_2O_3*, D	Fe_2O_4* 8.45, α-Fe_2O_3*, S	Fe_3O_4, 8.45, α-Fe_2O_3, S	Cr_2O_3, S
Refractory						
K42B	600	5	α-Fe_2O_3, D	Fe_3O_4, 8.43, S	Fe_3O_4, 8.43, S	Cr_2O_3, Fe_3O_4, 8.36, S
K42B	600	30	α-Fe_2O_3, D	Fe_3O_4, 8.44, α-Fe_2O_3, S	Fe_3O_4, 8.44, α-Fe_2O_3, S	Cr_2O_3, Fe_3O_4*, S
Inconel	600	5	Fe_3O_4, VD	Fe_3O_4, 8.43, S	Fe_3O_4, 8.43, S	Spinel, 8.32, Cr_2O_3, Ni, OD
Inconel	600	30	Fe_3O_4, VD	Fe_3O_4, 8.43, S	Fe_3O_4, 8.43, S	Cr_2O_3, NiO, VDO
Nichrome V	600	5	Cr_2O_3, VD	Cr_2O_3, S	Cr_2O_3, S	Cr_2O_3, S
Nichrome V	600	30	Cr_2O_3, VD	Cr_2O_3, S	Cr_2O_3, S	Cr_2O_3, M
Magnetic						
Mild steel	250	5	None	Fe_3O_4, 8.43, DO	Fe_3O_4, 8.43, DO	Fe_3O_4, 8.36, M
Mild steel	250	30	None	Fe_3O_4, 8.42, M	Fe_3O_4, 8.42, M	Fe_3O_4, 8.39, VS
Mild steel	300	5	Fe_3O_4, 8.43, α-Fe_2O_3, DO	Fe_3O_4, 8.43, α-Fe_2O_3, MO	Fe_3O_4, 8.43, α-Fe_2O_3, MO	Fe_3O_4, 8.41, SO
30 CoFe	300	30	Fe_3O_4, VD	Fe_3O_4, 8.43, M	Fe_3O_4, 8.43, M	Fe_3O_4, 8.36, M
Hipernik	300	5	Fe_3O_4, MO	Fe_3O_4, 8.42, α-Fe_2O_3, S	Fe_3O_4, 8.42, α-Fe_2O_3, S	Spinel, 8.34, α-Fe_2O_3, NiO, SO
Sealing						
Kovar	400	5	Fe_3O_4, 8.43, M	Fe_3O_4, 8.43, SO	Fe_3O_4, 8.43, SO	Fe_3O_4, 8.35, S
Miscellaneous						
5 CrFe	400	5	Fe_3O_4, DO	Fe_3O_4, 8.43, Cr_2O_3, *S	Fe_3O_4, 8.43, S	Fe_3O_4, or FeO.Cr_2O_3, 8.34, DO
5 NiFe	300	5	None	Fe_3O_4, 8.43, α-Fe_2O_3, M	Fe_3O_4, 8.43, α-Fe_2O_3, M	Fe_3O_4, 8.34, M
5 CoFe	300	5	Fe_3O_4, 8.43, M	Fe_3O_4, 8.43, M	Fe_3O_4, 8.43, S	Fe_3O_4, 8.35, SO
5 MnFe	300	10	α-Fe_2O_3, VD	Fe_3O_4, 8.43, S	Fe_3O_4, 8.43, S	Fe_3O_4, 8.32, S
5 SiFe	300	5	None	Fe_3O_4, 8.43, D O	Fe_3O_4, 8.43, DO	α-Fe_2O_3, M
3 VFe	300	5	Fe_3O_4, 8.42, D	Fe_3O_4, 8.42, D	Fe_2O_4, 8.42, M	Fe_3O_4, 8.33, DO
4 WFe	300	5	Fe_3O_4, VDO	α-Fe_2O_3, S	α-Fe_2O_3, S	α-Fe_2O_3, M

R, reflection. T, transmission. S, sharp. M, medium. D, diffuse. O, oriented. V, very. *, trace.

of the more typical shapes in the pattern. Uniformity in film thickness refers to the presence of thick and thin portions of the film. The type of pattern refers to a number of features, including: (1) the sharpness of the crystal edges, (2) the presence of overlapping crystals, and (3) the presence of extraneous material. These features are of importance in classifying a micrograph but may not be easily interpreted.

Light Microscope. Light micrographs of the oxidized surface are taken at 100× and 1000× by the use of reflected light. The oxidation process acts like a chemical etching solution in revealing the grain boundaries of the grains in the metal or alloy surface. The light micrographs also reveal the presence of inclusions and the roughness of the surface layer.

Spectroscopic Analysis. Whenever possible, sections of the stripped films were analyzed spectroscopically. In some cases these analyses showed the presence of metals not found in the alloys but present in the oxides used in the polishing procedures.

Table IV. Lattice Parameter Deviations of Oxides

Alloy	Temp. ° C.	Time of Oxidation Min.	Reflection Patterns: Composition and parameter	Deviation %	Transmission Patterns: Composition and parameter	Deviation %
Protective						
13 CrFe	600	5	Cr_2O_3	+0.30	Cr_2O_3	+0.04
	600	5	....	...	Fe_3O_4, 8.41	+0.12
18-8 SS	600	5	α-Fe_2O_3	+0.52	....	....
	600	5	Fe_3O_4, 8.44	+0.48	....	....
	600	5	....	...	Cr_2O_3	−0.02
18-8 SS	600	40	α-Fe_2O_3	+0.30	....	....
	600	40	Fe_3O_4, 8.45	+0.60	....	....
	600	40	....	...	Cr_2O_3	00.0
Refractory						
K42B	600	5	Fe_3O_4, 8.43	+0.36	Fe_3O_4, 8.36	−0.48
	600	5	....	...	Cr_2O_3	+0.07
K42B	600	30	Fe_3O_4, 8.44	+0.48	....	....
	600	30	α-Fe_2O_3	+0.32	....	....
	600	30	....	...	Cr_2O_3	+0.07
Inconel	600	5	Fe_3O_4, 8.43	+0.36	Spinel, 8.32	....
	600	5	....	...	Cr_2O_3	−0.11
	600	5	....	...	NiO	Diffuse
Inconel	600	30	Fe_3O_4, 8.43	+0.36	....	....
	600	30	....	...	Cr_2O_3	Too diffuse
	600	30	....	...	NiO	Too diffuse
Nichrome V	600	5	Cr_2O_3	+0.25	Cr_2O_3	−0.04
	600	30	Cr_2O_3	+0.30	Cr_2O_3	+0.04
Magnetic						
Mild steel	250	5	Fe_3O_4, 8.43	+0.36	Fe_3O_4, 8.36	−0.48
Mild steel	250	30	Fe_3O_4, 8.42	+0.24	Fe_3O_4, 8.39	−0.12
Mild steel	300	5	Fe_3O_4, 8.43	+0.36	Fe_3O_4, 8.41	+0.12
	300	5	α-Fe_2O_3	+0.25	....	....
30 CoFe	300	30	Fe_3O_4, 8.43	+0.36	Fe_3O_4, 8.36	−0.48
Hipernik	300	5	Fe_3O_4, 8.42	+0.24	Spinel, 8.34	−0.72
	300	5	α-Fe_2O_3	+0.10	α-Fe_2O_3	−0.03
	300	5	....	...	NiO	0.00
Sealing						
Kovar	400	5	Fe_3O_4, 8.43	+0.36	Fe_3O_4, 8.35	−0.60
Miscellaneous						
5 CrFe	400	5	Fe_3O_4, 8.43	+0.36	Fe_3O_4, 8.34	−0.72
5 NiFe	300	5	Fe_3O_4, 8.43	+0.36	Fe_3O_4, 8.34	−0.72
	300	5	α-Fe_2O_3	+0.25	....	....
5 CoFe	300	5	Fe_3O_4, 8.43	+0.36	Fe_3O_4, 8.35	−0.60
5 MnFe	300	10	Fe_3O_4, 8.43	+0.36	Spinel, 8.32	−0.96
5 SiFe	300	5	Fe_3O_4, 8.43	+0.36	....	....
5 SiFe	300	5	....	...	α-Fe_2O_3	0.00
3 VFe	300	5	Fe_3O_4, 8.42	+0.24	Spinel, 8.33	−0.84
4 WFe	300	5	α-Fe_2O_3	+0.16	α-Fe_2O_3	+0.08

RESULTS

Electron Diffraction. The results of the electron diffraction study are shown in Table III. Three reflection and one transmission patterns are taken of the oxide film formed on the surface of the alloy. The first reflection pattern of the metal is taken in the vacuum of the camera before oxidation and at the temperature of the experiment. The second is taken after the oxidation and at the temperature of the experiment. The third is taken after cooling the oxidized sample to 25° C. in a vacuum. The transmission pattern of the stripped oxide film is also included in order to compare the body structure of the film with its surface structure. Table III shows the conditions of oxidation, the chemical structure of the surface oxide, the unit cell size (A_0) where readily calculable, and the type of diffraction pattern obtained.

Let us consider the oxide film formed on mild steel at 250° C. and 0.1 atmosphere of oxygen with an oxidation time of 5 minutes. A diffuse and oriented pattern of Fe_3O_4 is found by reflection. The unit cell size is calculated to be 8.43 Å. After

Table V. Summary of Electron Diffraction and Spectrographic Data

Alloy	Oxidizing Conditions Temp. °C.	Time Min.	Composition and Parameter: Transmission	Reflection	X-ray data (literature)	Spectrographic data
Protective						
13 CrFe	600	5	Fe_3O_4*, 8.41, Cr_2O_3	Cr_2O_3	Fe_3O_4, 8.40	Cr, Fe, Ag*
18-8 SS	600	5	Cr_2O_3	Fe_3O_4*, 8.44, α-Fe_2O_3	Fe_3O_4, 8.40	.....
18-8 SS	600	40	Cr_2O_3	Fe_3O_4, 8.45, α-Fe_2O_3	Fe_3O_4, 8.40	.....
Refractory						
K42B	600	5	Fe_3O_4, 8.36, Cr_2O_3	Fe_3O_4, 8.43	Fe_3O_4, 8.40	Cr, Fe* (Mg)
K42B	600	30	Fe_3O_4*, Cr_2O_3	Fe_3O_4, 8.44, α-Fe_2O_3	Fe_3O_4, 8.40	.....
Inconel	600	5	Spinel, 8.32, Cr_2O_3, NiO	Fe_3O_4, 8.43	Fe_3O_4, 8.40, NiO.Cr_2O_3, 8.31	Cr, Ni, Fe (Al, Si, Co)
Inconel	600	30	Cr_2O_3, NiO	Fe_3O_4, 8.43	Fe_3O_4, 84.0	
Nichrome V	600	5	Cr_2O_3	Cr_2O_3	....	Cr, Fe* (No Ni)
Nichrome V	600	30	Cr_2O_3	Cr_2O_3	....	Cr, Fe* (No Ni)
Magnetic						
Mild steel	250	5	Fe_3O_4, 8.36	Fe_3O_4, 8.43	Fe_3O_4, 8.40	.....
Mild steel	250	30	Fe_3O_4, 8.39	Fe_3O_4, 8.42	Fe_3O_4, 8.40	.....
Mild steel	300	5	Fe_3O_4, 8.41	Fe_3O_4, 8.43, α-Fe_2O_3	Fe_3O_4, 8.40	.....
Hipernik	400	5	Spinel, 8.34, α-Fe_2O_3, NiO	Fe_3O_4, 8.42, α-Fe_2O_3	Fe_3O_4, 8.40, NiO.Fe_2O_3, 8.34	Mn, Ni, Fe*
30 CoFe	300	30	Spinel, 8.36	Fe_3O_4, 8.43	Fe_3O_4, 8.40, CoO.Fe_2O_3, 8.39	Fe, Co, (Cr*)
Sealing						
Kovar	400	5	Fe_3O_4, 8.35	Fe_3O_4, 8.43	Fe_3O_4, 8.40	.....
Miscellaneous						
5 CrFe	400	5	Spinel, 8.34	Fe_3O_4, 8.43	Fe_3O_4, 8.40, FeO.Cr_2O_3, 8.35	Cr, Fe*
5 NiFe	300	5	Spinel, 8.34	Fe_3O_4, 8.43	Fe_3O_4, 8.40, NiO.Fe_2O_3, 8.34	.....
5 CoFe	300	5	Spinel, 8.35	Fe_3O_4, 8.43	Fe_3O_4, 8.40, CoO.Fe_2O_3, 8.39	Fe, Cu, (Al*), No, Co
5 MnFe	300	10	Spinel, 8.32	Fe_3O_4, 8.43	Fe_3O_4, 8.40, MnO.Fe_2O_3, 8.51	Fe, Mn, (Cr, Al*)
5 SiFe	300	5	α-Fe_2O_3	Fe_3O_4, 8.43	Fe_3O_4, 8.40	.....
3 VFe	300	5	Spinel, 8.33	Fe_3O_4, 8.42	Fe_3O_4, 8.40	.....
4 WFe	300	5	α-Fe_2O_3	α-Fe_2O_3		

* Trace.

deviations of the oxides formed in each oxidation experiment are shown in Table IV. This table shows the conditions of oxidation, the composition, and unit cell size where readily calculable and the deviation in per cent from the accepted x-ray values given in Table II. Both the reflection and transmission patterns are tabulated. The deviations are calculated from the unit cell size as given in Table III or from the d/N values for those oxides which do not obey the cubic lattice.

A summary of the electron diffraction and spectrographic data obtained on the 16 alloys is shown in Table V. Both the transmission and reflection data are given, together with the x-ray data obtained from the literature.

Electron Microscope. Figures 1 to 11 show the light micrographs, electron micrographs, electron diffraction transmission, and electron diffraction reflection patterns for the stripped oxide films from the alloy specimens. The lengths of 1, 10, or 100 microns are shown on the photographs. The light micrographs of the unstripped oxide film are taken at 100 and 1000×, while the electron micrographs of the stripped oxide film are taken at 6800× and enlarged optically to 34,000×. These micrographs are reduced subsequently in the printing process. The actual magnifications can be readily calculated from the fact that each centimeter of the length of the micron line shown in the micrograph equals 10,000×.

oling the sample to room temperature, the oxide film is stripped om the metal. A medium pattern of Fe_3O_4 is found using the ansmission method on the stripped film. The unit cell size is lculated to be 8.36 Å. Figure 10, *b* and *c*, shows the electron difaction photographs for this oxidation experiment. In a similar anner the results of the other experiments on mild steel and the fteen other alloys are shown in Table III and Figures 1 to 11.

In general the lattice parameters obtained in this study deviate om the accepted x-ray diffraction values. The lattice parameter

Table VI. Electron Microscope Analyses of Stripped Oxide Films of Alloys

Alloy	Oxidizing Conditions 0.1 Atmosphere of O_2 Min.	°C.	Film Color on Metal	Figure	Particle Size, Å.	Size Distribution, Å.	Shape	Uniformity	Type of Micrograph
Protective									
13 CrFe	5	600	Blue	1	450	200 to 700	Irregular	Nonuniform	Medium, overlapping crystals
18-8 SS	5	600	Blue and brown	2	300	100 to 600	Irregular	Uniform	Medium
	40	600	Yellow and blue	3	700	300 to 1500	Irregular	Nonuniform	Sharp, overlapping crystals
Refractory									
K42B	5	600	Yellow and mauve	4	350	200 to 750	Irregular	Nonuniform	Medium, clusters of crystals
	30	600	Reddish blue	..	450	300 to 800	Irregular	Nonuniform	Sharp, clusters of crystals
Inconel	5	600	Light blue	5	400	300 to 800	Irregular	Nonuniform	Medium, overlapping crystals, grain boundaries show
Inconel	30	600	Red	..	450	300 to 900	Irregular	Nonuniform	Medium, overlapping crystals
Nichrome V	5	600	Light blue	6	250	100 to 450	Irregular	Fairly uniform	Medium, overlapping crystals
	30	600	Yellow	..	350	200 to 550	Irregular	Fairly uniform	Medium, overlapping crystals
Magnetic									
Mild steel	5	250	Dark blue	7	700	300 to 1600	Irregular	Nonuniform	Medium overlapping crystals
	30	250	Dark blue	..	800	400 to 1200	Irregular	Nonuniform	Medium, overlapping crystals, striations
	5	300	Light blue, hiatus	..	750	250 to 900	Irregular	Nonuniform	Medium, overlapping crystals
30 CoFe	30	300	Light mauve	8	700	300 to 1200	Irregular, angular	Nonuniform	Sharp, overlapping crystals
Hipernik	5	400	Blue	..	300	200 to 500	Irregular	Uniform, thicker at grain boundaries	Medium, overlapping crystals, grain boundaries show
Kovar	5	400	Pink and light blue	..	400	250 to 750	Irregular	Nonuniform	Medium, clusters of small crystals
Miscellaneous									
5 CrFe	5	400	Light blue	9	250	100 to 400	Irregular	Nonuniform	Medium, chains of clusters of crystals
5 NiFe	5	300	Yellow and mauve	..	600	250 to 1000	Irregular	Nonuniform	Medium, overlapping crystals
5 CoFe	5	300	Pink and blue	..	400	250 to 750	Irregular	Nonuniform	Diffuse, overlapping crystals, clusters of crystals
5 MnFe	10	300	Light blue, hiatus	10	350	250 to 600	Irregular	Uniform	Sharp, overlapping crystals
5 SiFe	5	300	Reddish-blue	11	500	250 to 750	Irregular	Uniform	Sharp, overlapping crystals
3 VFe	5	300	Mauve and blue	..	250	200 to 300	Irregular	Uniform	Medium, large clusters of crystals on fine grained matrix
4 WFe	5	300	Mauve	..	300	200 to 400	Irregular, indistinct	Fairly uniform	Diffuse, network of thin strips

Figure 1. Oxide Film of 13% Chrome-Iron, 13 Cr 5–600

a. Electron micrographs, stripped film
b. Electron diffraction transmission, stripped film
c. Electron diffraction reflection, film on metal
d, *e*. Light micrographs, film on metal

Table VI summarizes the information recorded from the electron microscope: (1) color of the oxide film on the metal, (2) particle size in Ångströms, (3) particle size distribution, (4) particle shape, (5) film uniformity, and (6) type of micrograph.

DISCUSSION

The factors which determine the chemical and physical structure of the oxide film on the surface of an alloy have been discussed in a previous paper (*10*):

1. Rates of formation and diffusion of the various metal ions and electrons through the oxide lattice
2. The rate of diffusion of the oxygen molecule, ion or atom through the oxide lattice
3. Thermodynamic stabilities of the oxides formed
4. Lattice type and its resemblance to the original metal or alloy lattice
5. Chemical reactions occurring in the oxide film between the several oxides or between metal ions or oxygen atoms and the several oxides
6. Preoxidation treatment given to the alloy, such as annealing, polishing, and cleaning
7. Rate of nucleation and growth of the oxide crystal

Factors 3 and 4 are known from thermodynamic and lattice structure data, while factor 5 can be estimated from thermodynamic data. Factors 1, 2, and 7 are unknown from both theory and experiment. Some evidence is available experimentally on factor 6. In a previous paper (*8*) the authors studied the effect of abrasion treatment on the surface oxide films formed on iron at high temperatures. Vernon, Wormwell, and Nurse (*17*) have studied the effect of polishing procedures on the oxide films formed on 18-8 stainless steel.

An analysis of the factors listed above would show that on alloys one might expect to obtain, after oxidation, reaction products which concentrate one or more of the oxides at the expense of the other components in the alloy. This has been shown in a previous paper (*10*). Thus NiO is never observed on the surface in the oxidation of its alloys containing up to 80% nickel. Cr_2O_3 and Fe_3O_4 or γ-Fe_2O_3, however, are observed on alloys where chromium and iron may be present in amounts less than 5%.

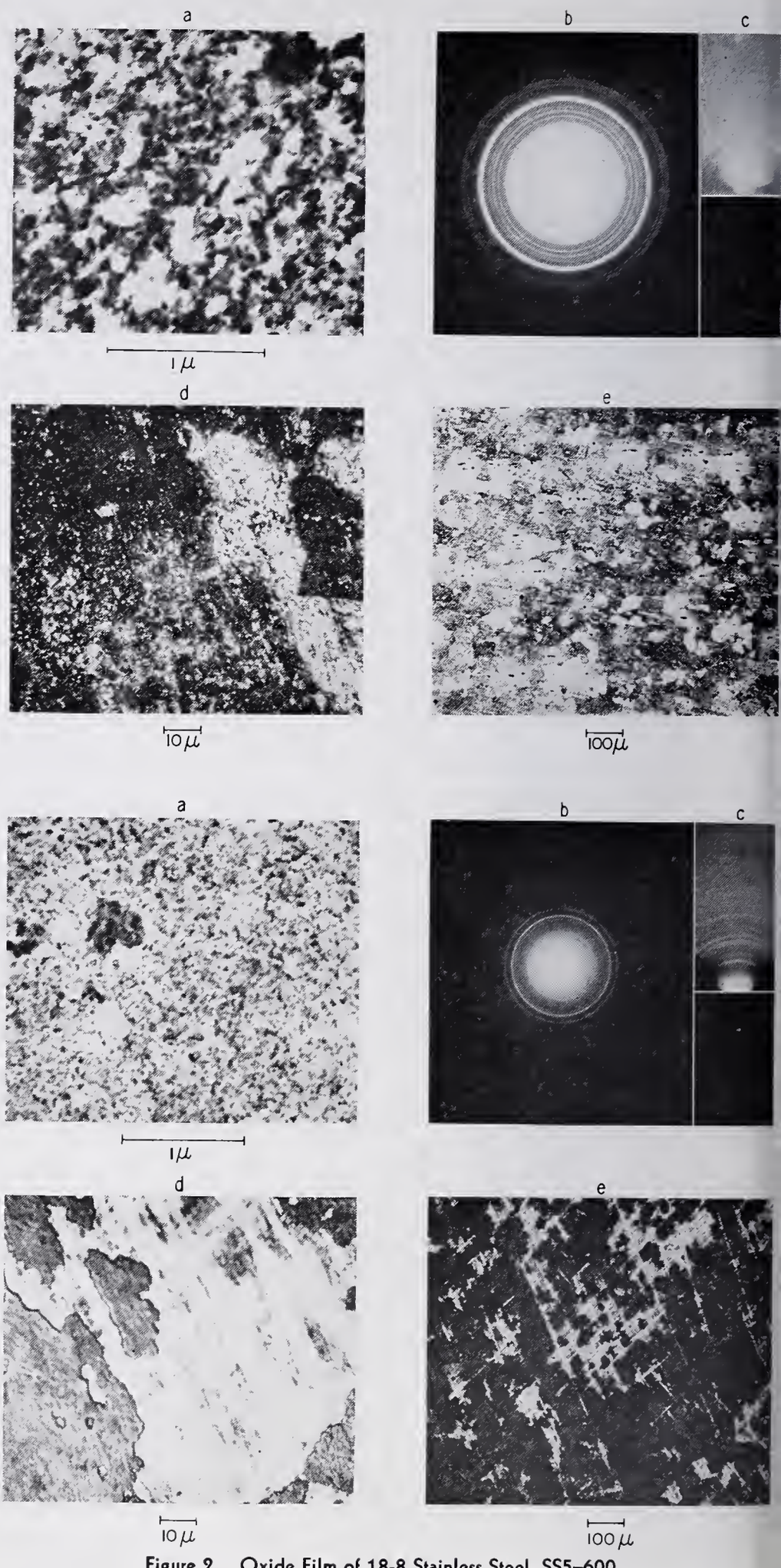


Figure 2. Oxide Film of 18-8 Stainless Steel, SS5–600

a. Electron micrographs, stripped film
b. Electron diffraction transmission, stripped film
c. Electron diffraction reflection, film on metal
d, *e*. Light micrographs, film on metal

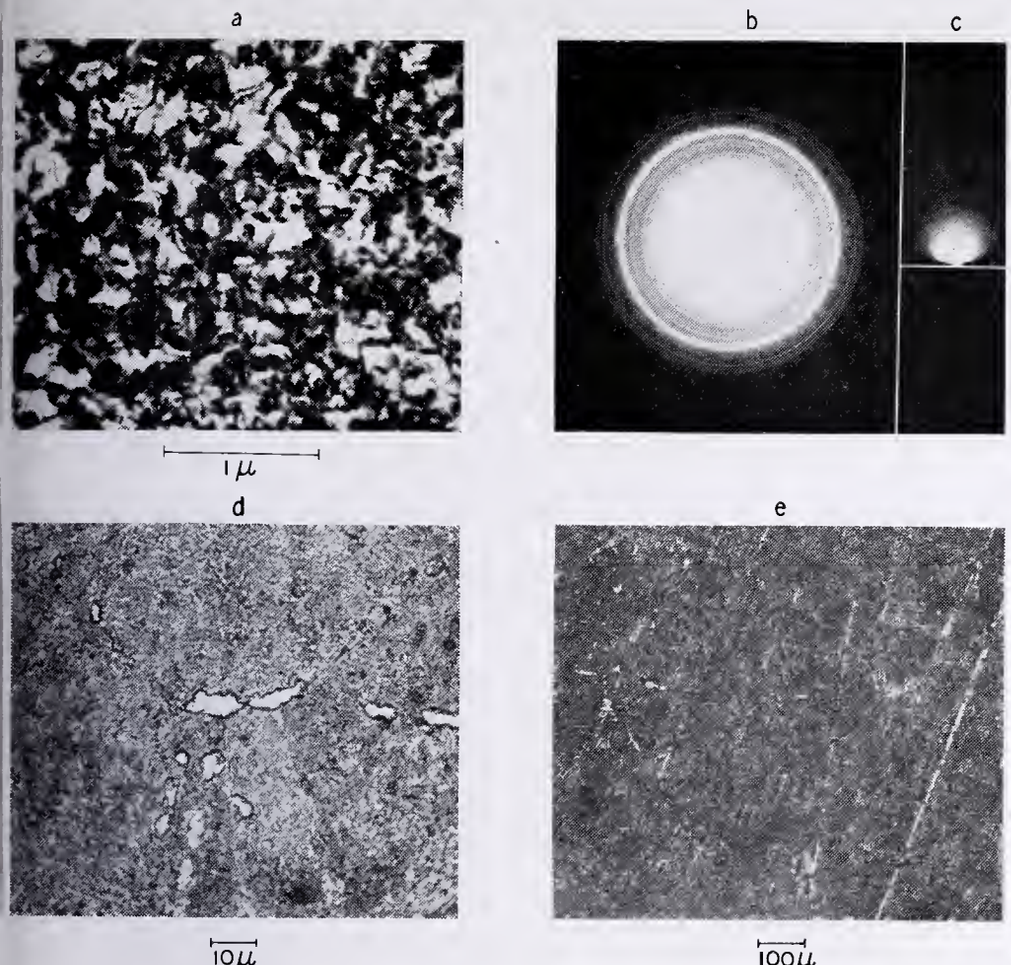


Figure 3. Oxide Film of 18-8 Stainless Steel, SS40–600

a. Electron micrographs, stripped film
b. Electron diffraction transmission, stripped film
c. Electron diffraction reflection, film on metal
d, e. Light micrographs, film on metal

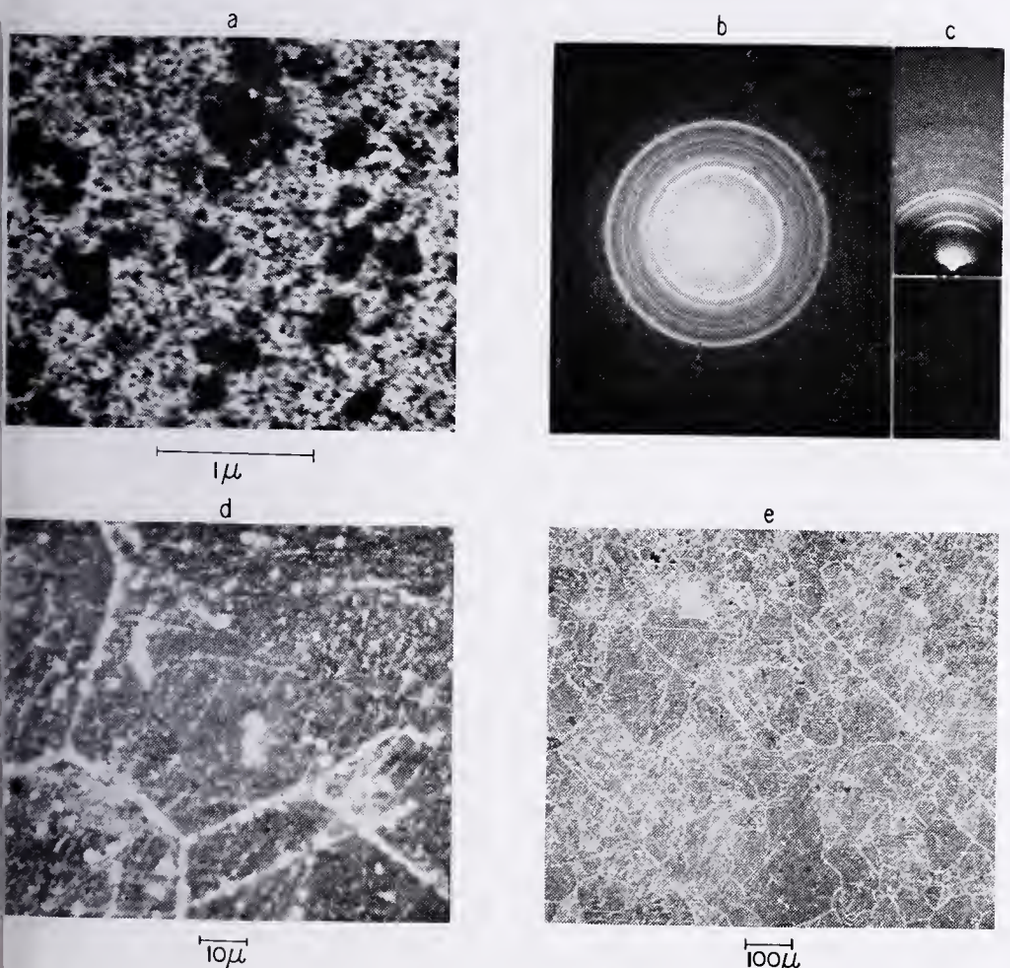


Figure 4. Oxide Film of K42B, K5–600

a. Electron micrograph, stripped film
b. Electron diffraction transmission, stripped film
c. Electron diffraction reflection, film on metal
d, e. Light micrographs, film on metal

The use of the stripping technique to remove the oxide film and a study of the stripped oxide film by the transmission method should show further evidence, when compared with reflection measurements, of the concentration of the several components in the oxide film.

A previous electron microscope study (*15*) of the stripped oxide films formed on iron, cobalt, nickel, tungsten, chromium, molybdenum, columbium, aluminum, and copper has shown that the films consist of small oxide crystals of 100 to 2500 Å. in size. The films consist largely of crystals of irregular shapes, although a few films show definite crystal outlines. The oxide crystals are of the order of 10^{-3} to 10^{-5} of the linear dimension of the metal crystal or grain and 10^{-6} to 10^{-10} of the area.

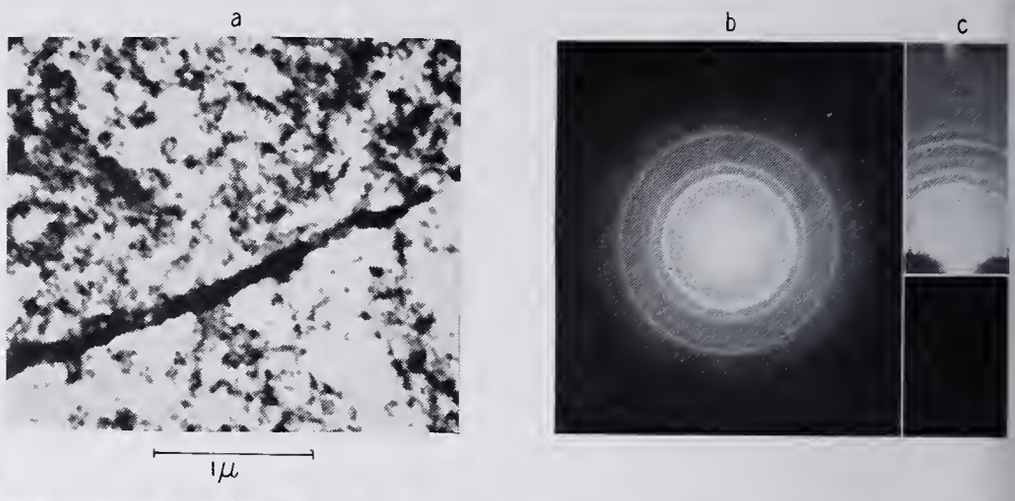
A systematic study of stripped oxide films on metals is difficult using electron microscope techniques. This is due to several factors: (1) The limitation imposed on the sample by the electron microscope—i.e., the thickness range of the specimen is limited to samples of about 100 to 500 Å. (2) The limitation in the resolving power of the electron microscope; with a resolving power of 40 Å. it is difficult to determine the shapes and the nature of the boundaries of crystals 100 to 200 Å. in size. (3) The scattering and diffraction of the electrons by the sample. If diffraction effects occur at grain boundaries, the nature of the boundary zone may not be determined.

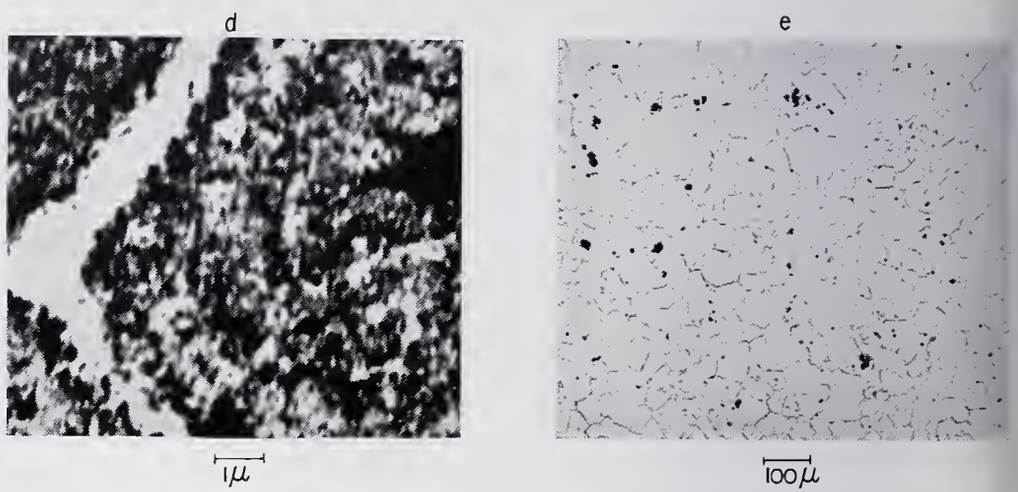
The study on alloys is further complicated in some cases by the presence of several oxides in the film. An examination of Table V shows that mixtures of oxides occur on 13 CrFe, K42B, Inconel, and Hipernik.

In the previous study (*15*) it was proposed that nucleation and growth of crystals had resulted in the formation of a mosaic structure completely covering the surface. This was noticed even for films less than 75 Å. thick. It would appear that at no time is the metal or alloy surface exposed to the gas atmosphere after the formation of the first chemi-adsorbed layer of oxygen atoms. The transformation from the first chemi-adsorbed layer of oxygen atoms to the continuous layer of small crystals is a continuous process of nucleation

Figure 5. Oxide Film of Inconel, 5–600

a, d. Electron micrographs, stripped film
b. Electron diffraction transmission, stripped film
c. Electron diffraction reflection, film on metal
e. Light micrographs, film on metal





and growth. This continuous layer of small crystals is shown for the oxide film formed on silicon iron in Figure 11.

It is of interest to contrast this type of thin film with that formed by the evaporation of metals. Here the metal condenses into a discontinuous group of crystals. No evidence of a mosaic structure is noticed.

The continuous film of oxide crystals covering the surface of the metal or alloy may be the mechanism by which the property of protection is given to the metal or alloy. Since the chemi-adsorbed layer transforms readily to a continuous layer of crystals, a mechanical break in the film is readily repaired.

Protective Alloys. 13 CrFe and 18-8 stainless steel are classified as protective alloys, since they react only very slightly to oxygen atmospheres at moderate temperatures. Here a more quantitative concept is used in defining the term protective—i.e., a metal or alloy which obeys a parabolic oxidation rate law at a given temperature and pressure is considered to be protective. All the 16 alloys considered here are studied under conditions where the alloy follows the parabolic rate law.

13 CrFe. This alloy consists of a solid solution of chromium in the body-centered cubic lattice of α-iron. The oxide film formed at 600° C. in 0.1 atmosphere of oxygen for 5 minutes gives a pattern of Cr_2O_3 and in addition a trace of Fe_3O_4 using the transmission technique. Spectrographic analysis indicates the presence of chromium and iron as shown in Table V.

The electron microscope study of the stripped oxide film shown in Figure 1 and Table VI indicates a nonuniform film of irregularly shaped particles with a size distribution of 200 to 700 Å. while the average crystal size is about 450 Å.

Mott's theory of oxidation (*13*) would predict the occurrence of oxides of iron on the surface and Cr_2O_3 in contact with the substrate.

18-8 Stainless Steel. This alloy consists of a solid solution of chromium and nickel in the face-centered cubic lattice of γ-iron. Two oxidation experiments are made at 600° C., the first for 5 minutes and the second for 40 minutes. A mixture of Fe_3O_4 and α-Fe_2O_3 is found by the reflection study, while Cr_2O_3 is the only oxide found in the stripped film using the transmission technique. No spectrographic analyses were made. The evidence indicates that oxides of iron are con-

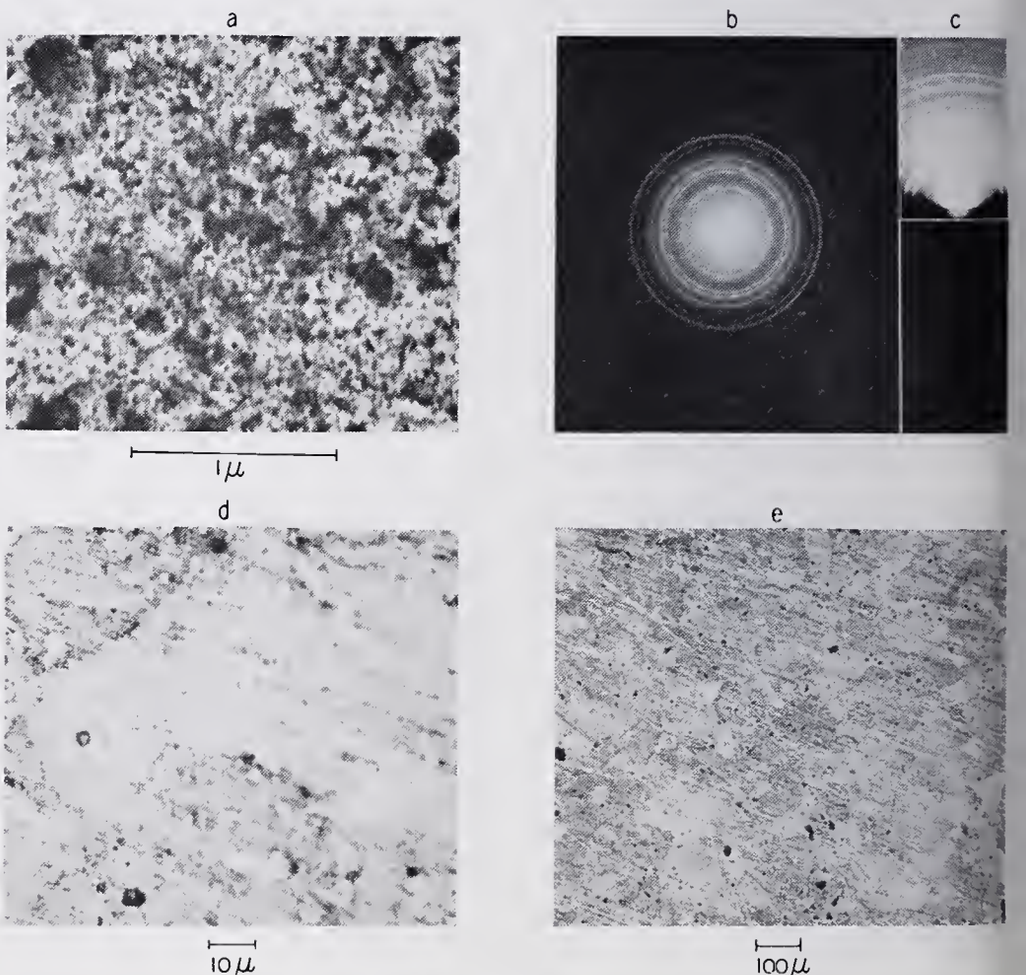
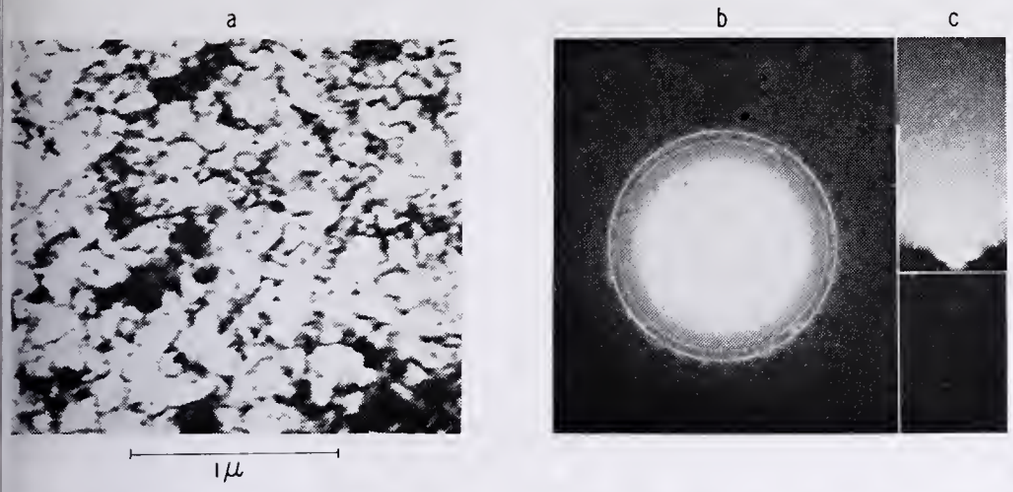


Figure 6. Oxide Film of Nichrome V, NC5–600

a. Electron micrographs, stripped film
b. Electron diffraction transmission, stripped film
c. Electron diffraction reflection, film on metal
d, e. Light micrographs, film on metal



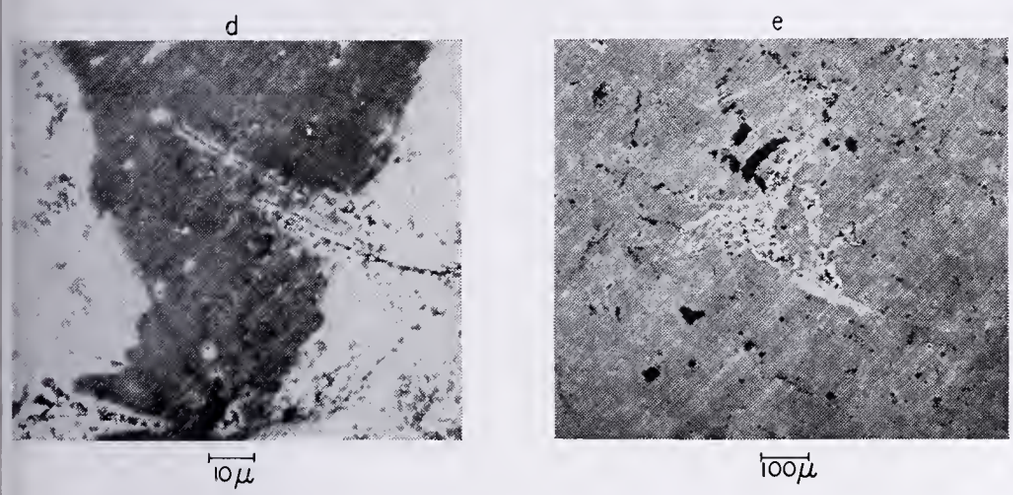
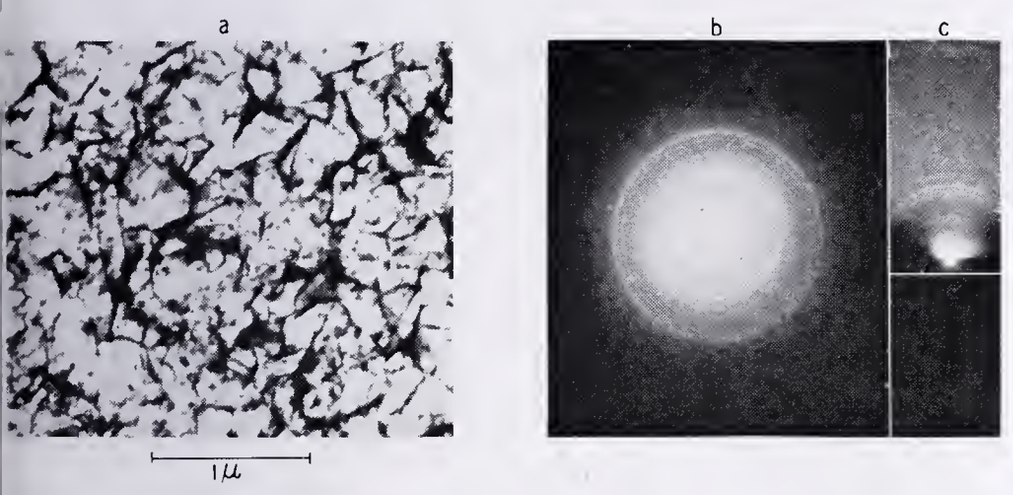


Figure 7. Oxide Film of Mild Steel, MS5--250

a. Electron micrographs, stripped film
b. Electron diffraction transmission, stripped film
c. Electron diffraction reflection, film on metal
d, e. Light micrographs, film on metal



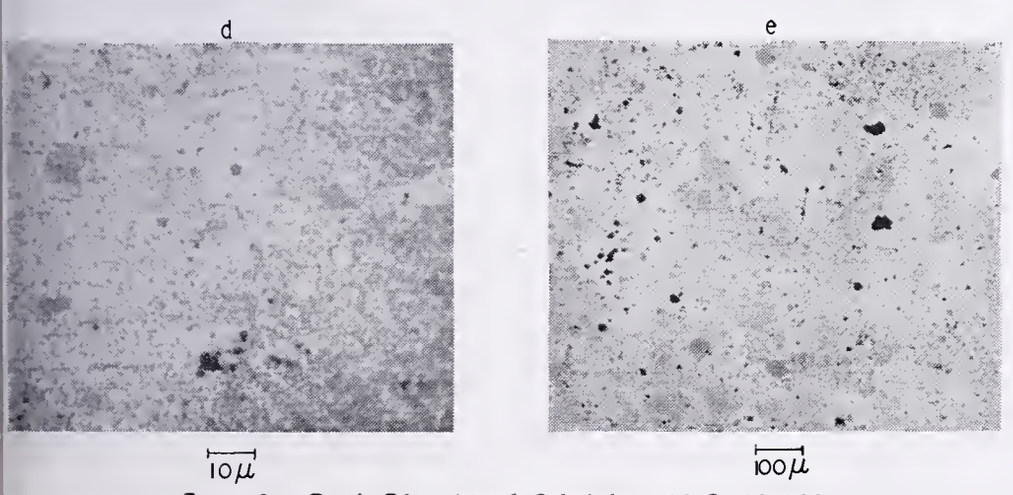


Figure 8. Oxide Film of 30% Cobalt-Iron, 30 Co 30–300

a. Electron micrographs, stripped film
b. Electron diffraction transmission, stripped film
c. Electron diffraction reflection, film on metal
d, e. Light micrographs, film on metal

centrated in the outer part of the film, while Cr_2O_3 is the main component of the film. These results, when compared with those obtained on 13 CrFe as shown in Table VI, indicate that the presence of nickel in the lattice has had an effect on the relative rates with which chromium and iron get to the surface.

The electron microscope data shown in Table VI and Figures 2 and 3 indicate that small, irregular crystals of 100 to 600 Å. are formed for the 5-minute oxidation while somewhat larger, irregular crystals of 300 to 1500 Å. are formed in the 40-minute oxidation. The average size increases with time of oxidation from 300 to 700 Å. as shown by the two experiments. The 5-minute oxidation shows a uniform film while the 40-minute oxidation shows a nonuniform film.

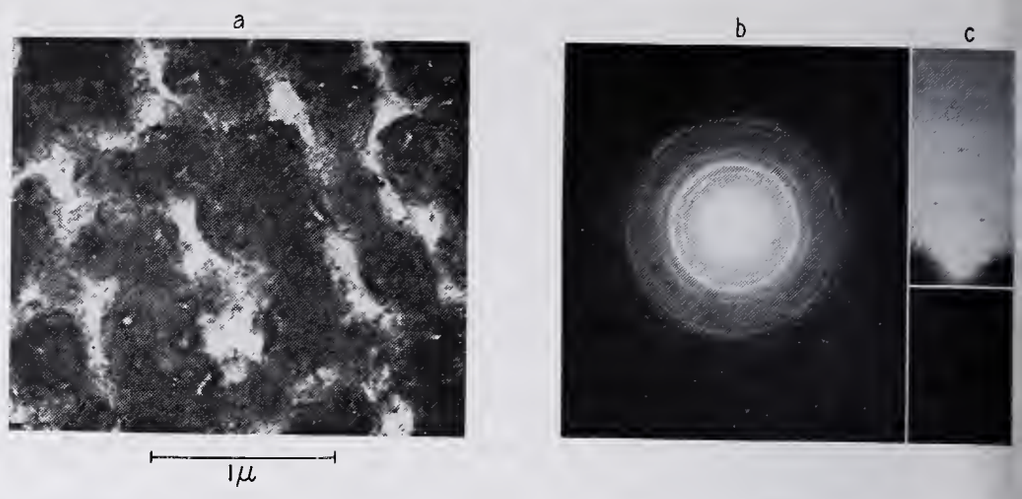
Refractory Alloys. These alloys are not only protective but exhibit great strength at high temperatures. They are usually complex chemically and in many cases are not structurally homogeneous.

K42B. K42B is a complex alloy and consists largely of a solid solution of the several metals in a face-centered cubic lattice. Two experiments are made with K42B at 600° C. The first oxidation is for 5 minutes, while the second is for 30 minutes. The reflection electron diffraction data shown in Tables III and V indicate a spinel structure on the surface which the authors have assigned the formula Fe_3O_4, because of its lattice parameter. The 30-minute oxidation study indicates the presence of α-Fe_2O_3 in addition to Fe_3O_4 on the surface. This is to be expected from previous observations on iron by the authors (*8*) where α-Fe_2O_3 is formed under conditions of an unlimited oxygen supply and long oxidation time. The transmission diffraction patterns show the presence of Cr_2O_3 and a spinel, while spectrographic analysis shows both chromium and iron with chromium making up the bulk of the film. It is of interest that stratification occurs even in films 100 to 300 Å. in thickness. Since the 30-minute oxidation shows only a trace of Fe_3O_4 by transmission while the reflection data indicate that the surface consists of oxides of iron, Cr_2O_3 appears to be in contact with the substrate. This is in agreement with results for stainless steel and in contrast with those for 13 CrFe.

The electron microscope data are summarized in Table VI and shown in Figure 4. Nonuniform films of irregular crystals are formed in both experiments. Crystals from 200 to 750 Å. in size, with an average size of 350 Å., are found for

Figure 9. Oxide Film of 5% Chrome-Iron, 5 Cr 5–400

a. Electron micrographs, stripped film
b. Electron diffraction transmission, stripped film
c. Electron diffraction reflection, film on metal
d, *e*. Light micrographs, film on metal



the 5-minute oxidation, while the size distribution is 300 to 800 Å. and the average size 450 Å. for the 30-minute experiment. The average crystal size increases with an increase in the time of oxidation.

Inconel. This alloy is probably a solid solution type of alloy with a face-centered cubic lattice. Two experiments are made at 600° C.; the first oxidation is for 5 minutes, while the second is for 30 minutes.

The reflection studies indicate the presence of Fe_3O_4 for both experiments. A transmission study of the stripped film shows the presence of a spinel, Cr_2O_3, and NiO for the 5-minute oxidation, and Cr_2O_3 and NiO for the 30-minute oxidation. Spectrographic analysis shows the presence of chromium, nickel, and iron in the film.

An analysis of these results indicates that a stratification of the oxides occurs in the film formed on Inconel with a spinel, probably Fe_3O_4, on the surface and a spinel, probably $NiO.Cr_2O_3$, and Cr_2O_3 and NiO making up the body of the film. The data available do not permit a determination of the relative positions of the Cr_2O_3 and NiO in the film. It is interesting to note that NiO and Cr_2O_3 can exist together in the film without forming completely the spinel $NiO.Cr_2O_3$. An x-ray diffraction study of the equilibrium system Cr_2O_3-NiO by Thomassen (*16*) is shown in Figure 12. No part of the chart shows the existence of NiO and Cr_2O_3 except as the spinel $NiO.Cr_2O_3$.

The electron microscope data are summarized in Table VI and the micrograph is shown in Figure 5. Nonuniform oxide films are obtained with irregularly shaped crystals. The crystals vary in size from 300 to 900 Å. with an average size of 400 to 450 Å. An increase in the time of oxidation does not result in an appreciable increase in average crystal size.

Nichrome V. Nichrome V is an alloy used in making electrical resistance heaters. It consists largely of a solid solution of chromium in nickel with a face-centered cubic lattice.

Two oxidations are made, the first for 5 and the second for 30 minutes at 600° C. The electron diffraction data as summarized in Tables III and V show that Cr_2O_3 is found by both the reflection and transmission methods. This is in agreement with a previous study (*9*) on the existence diagram for Nichrome V. Mott's theory (*13*) would, however, predict the occurrence of NiO on the surface and Cr_2O_3 in contact with the substrate. Spectrographic analysis shows the presence of chromium and a trace of

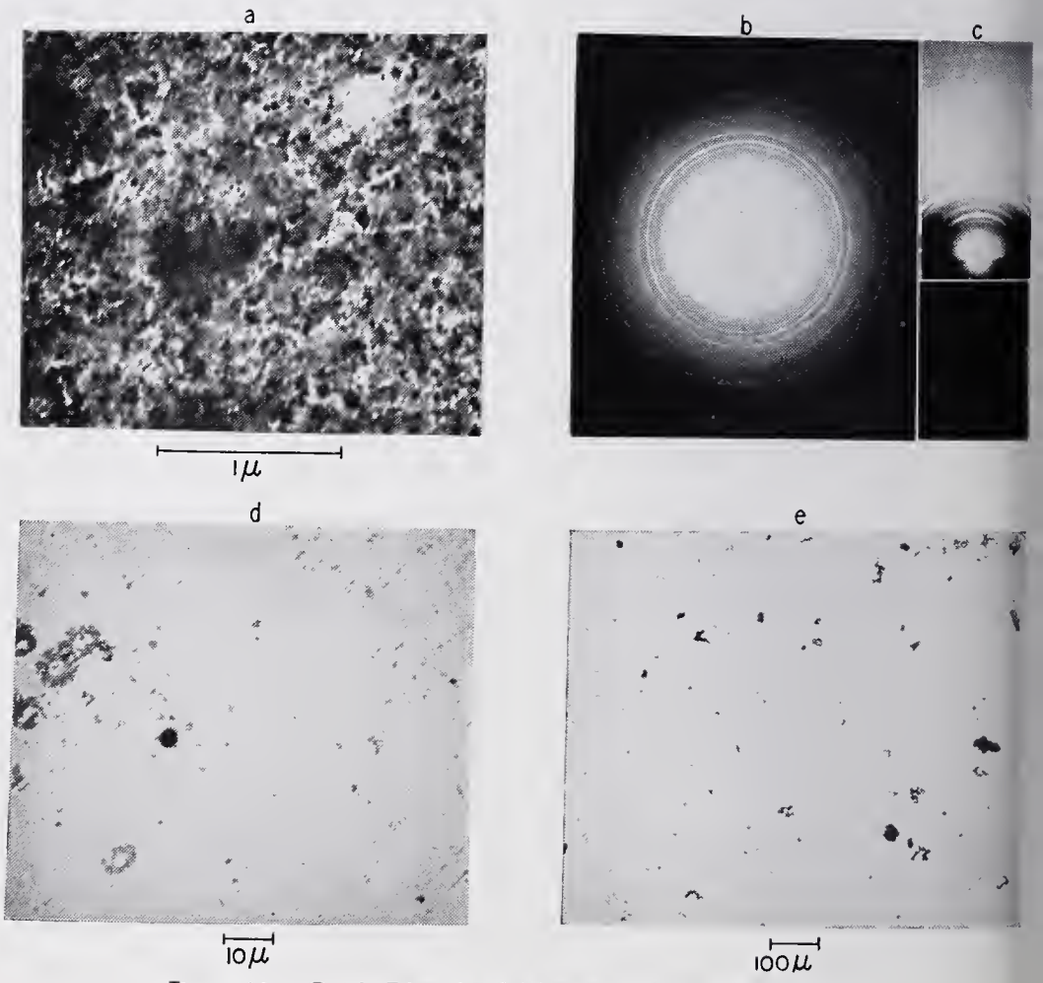


Figure 10. Oxide Film of 5% Manganese Iron, 5 Mn 10–300

a. Electron micrographs, stripped film
b. Electron diffraction transmission, stripped film
c. Electron diffraction reflection, film on metal
d, *e*. Light micrographs, film on metal

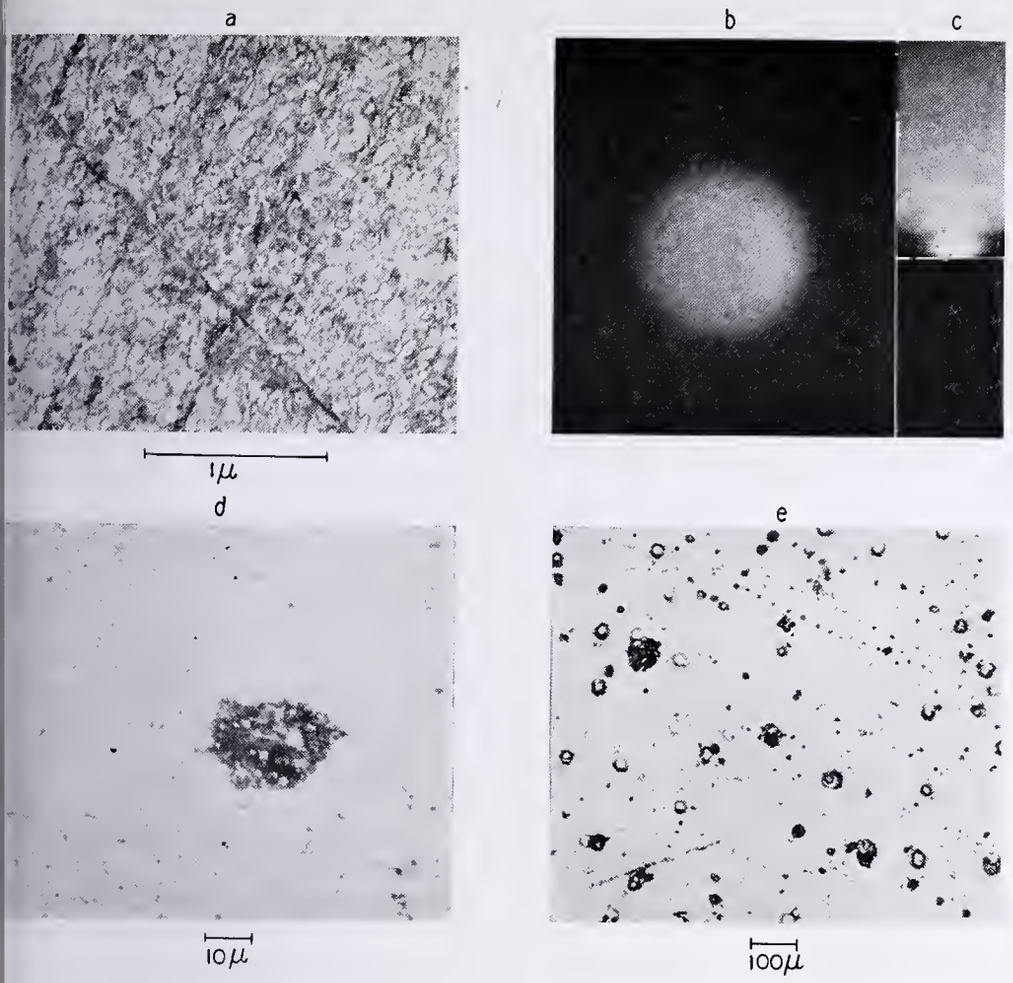


Figure 11. Oxide Film of 5% Silicon Iron, 5 Si 5–300

a. Electron micrographs, stripped film
b. Electron diffraction transmission, stripped film
c. Electron diffraction reflection, film on metal
d, e. Light micrographs, film on metal

The lattice parameters for the Cr_2O_3 lattice found in this study are shown in Figure 13, together with the other possible lattices which might be formed. The agreement with the Cr_2O_3 lattice is good.

Electron microscope data summarized in Table VI and the electron micrograph shown in Figure 6 indicate the presence of fairly uniform oxide films made up of irregularly shaped crystals. The crystals have a size distribution of 100 to 550 Å. with an average size of 250 Å. for the 5-minute oxidation and 350 Å. for the 30-minute oxidation. The time effect of size increase is small but can be noticed.

MAGNETIC ALLOYS. *Hipernik.* This alloy is of the solid solution type and has a face-centered cubic lattice. It is best known for its magnetic properties. It is an interesting alloy, since it consists of 49% iron, 49% nickel, and 2% manganese. In terms of the atomic concentration of the metals on the surface of the alloy, oxides of both iron and nickel should occur.

The oxide film formed at 400° C. in 0.1 atmosphere of oxygen for 5 minutes shows the presence of Fe_3O_4 and α-Fe_2O_3 on the surface by the reflection technique. The transmission data show the presence of a spinel with a lattice parameter of 8.34 Å. as well as α-Fe_2O_3 and NiO. The lattice parameter of the spinel does not permit a positive identification, since Fe_3O_4 has a parameter of 8.40 Å. and $NiO.Fe_2O_3$ has a parameter of 8.34 Å. Spectrographic analysis shows the presence of manganese, nickel, and a trace of iron.

The electron microscope data summarized in Table III indicate the presence of a uniform film with thicker sections at the grain boundaries. The crystals are irregular in shape and show evidence of overlapping. The crystal size varies from 200 to 500 Å. with an average size of 300 Å.

30 CoFe. This alloy is of the solid solution type and has a body-centered cubic structure. It is known for its magnetic rather than for its protective properties.

The oxide film formed at 300° C. in 0.1 atmosphere of oxygen for 30 minutes shows the presence of Fe_3O_4 (8.43 Å.) on the surface by reflection and a spinel (8.36 Å.) by transmission. The two spinels which are possible are Fe_3O_4 (8.40 Å.) and $CoO.Fe_2O_3$ (8.39 Å.). The body of the film may be either of these or a mixture of the two. Spectrographic analysis shows iron, cobalt, and a trace of chromium.

The electron microscope data given in Table III and the electron micrograph shown in Figure 8 indicate the presence of a nonuniform film composed of irregular, angular crystals with a size distribution of 300 to 1200 Å. and an average crystal size of 700 Å. Considerable overlapping of crystals is noted.

Mild Steel. Mild steel may be classified as a magnetic alloy with poor protective quality. It has a body-centered cubic structure.

Oxidations with 0.1 atmosphere of oxygen at 250° C. for 5 and 30 minutes show the presence of Fe_3O_4 by both techniques. Oxidation at 300° C. for 5 minutes shows Fe_3O_4 and α-Fe_2O_3 by reflection from the surface and Fe_3O_4 by transmission

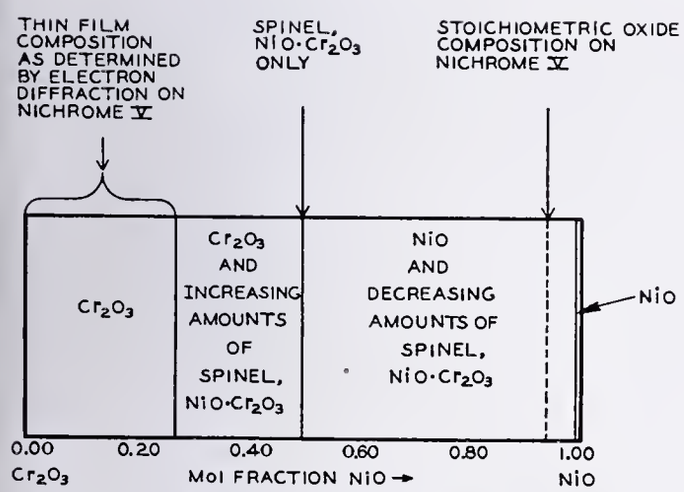


Figure 12. Correlation of Electron Diffraction Study with Equilibrium Diagram Study of System Cr_2O_3–NiO *(15)*

on but no nickel. The authors' data also differ from the results f Chalmers and Quarrell (*2*) who report that the oxide formed on ickel-chromium alloys has a spinel structure and probably a omposition corresponding to $NiO.Cr_2O_3$.

It is of interest to compare results for Nichrome V with the re-ılts of the equilibrium diagram for the Cr_2O_3–NiO system as etermined by Thomassen (*16*) and shown in Figure 12. If the lloy were completely converted to the oxides, the diagram shows ıat one should obtain NiO and the spinel $NiO.Cr_2O_3$. The au-ıors obtain from electron diffraction data only Cr_2O_3. Referring gain to Figure 12 one can see that Cr_2O_3 would be the equilibrium xide forming up to NiO mole fractions of 0.27, the NiO being resent in solid solution in the Cr_2O_3. This example illustrates the ifficulty of applying equilibrium data to a thin film reaction prod-ct forming on alloys.

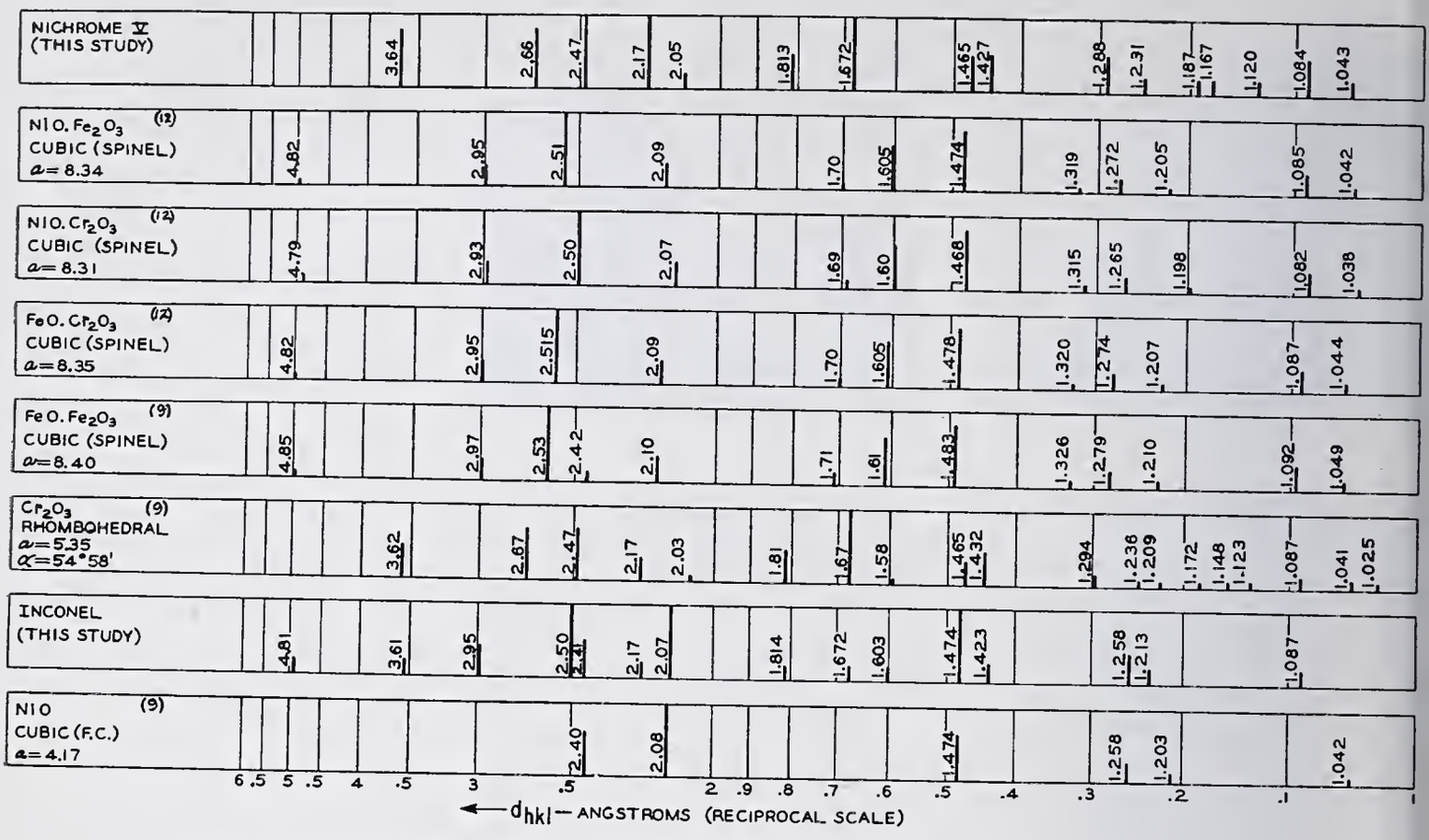


Figure 13. Experimental Diffraction Data for Oxides Which May Occur on Nichrome V and Inconel

The electron micrograph shown in Figure 7 indicates the presence of nonuniform film consisting of overlapping crystals with a size distribution of 250 to 1600 Å. and an average size of 750 Å. for the three oxidations. The size of the crystals does not appear to be affected by temperature.

Sealing Alloy. Kovar is a solid solution type of alloy having a face-centered cubic structure. It is used primarily in making glass to metal seals, and is not considered to be a protective alloy. However, the nature of the oxide film is very important in preparing good seals.

Oxidation with 0.1 atmosphere of oxygen at 400° C. for 5 minutes shows the presence of Fe_3O_4 by reflection and a spinel with lattice parameter of 8.35 Å by transmission. Since the parameter of $NiO.Fe_2O_3$ is 8.34 Å. while that of Fe_3O_4 is 8.40 Å. and $CoO.Fe_2O_3$ is 8.39 Å., it would appear that the nickel spinel may be formed. Spectrographic tests were not made on this alloy and therefore a positive identification is impossible.

The electron micrograph indicates the presence of a nonuniform film consisting of clusters of small irregular crystals with a size distribution of 250 to 750 Å. and an average size of 400 Å.

Miscellaneous Alloys. These binary alloys are composed of iron and metals of the body-centered cubic, face-centered cubic, and other structural types in solid solution in the α-iron lattice. In all cases the alloying metal is not present in excess of 5%. The alloying metals tungsten, chromium, and vanadium have body-centered cubic structures; Nickel is face-centered cubic; cobalt is hexagonal close packed; Manganese has a complex cubic structure; and silicon has a cubic structure of the diamond type. Previous work by the authors (*10*) on abraded samples of these alloys indicated that oxides of the alloying metal were not found on the surface. This study has been undertaken to determine whether polishing may increase the concentration of the alloying metal to such an extent that its oxides may occur on the surface and to determine whether oxides of the alloying metal occur in the body of the film.

5 CrFe. Transmission data show the presence of a spinel with a lattice parameter of 8.34 Å. in the body of the film. Spectrographic analysis shows the presence of chromium and a trace of iron. This would indicate that $FeO.Cr_2O_3$ (8.35 Å.) may be the oxide in the body of the film with Fe_3O_4 on the surface. It may also be possible that γ-Fe_2O_3 (8.32 Å.) is present in the body of the film.

3 VFe. The transmission data show the presence of a spinel with a lattice parameter of 8.33 Å. Although a spinel with the formula $FeO.V_2O_3$ may exist, no x-ray data appear to be available for this substance. The body of the film may, therefore, be composed of this spinel or it may be γ-Fe_2O_3 (8.32 Å.). No spectrographic analysis is available to check these predictions.

5 NiFe. Although the surface spinel appears to be Fe_3O_4, the formula of the spinel occurring in the body of the film may be $NiO.Fe_2O_3$. The lattice parameter by transmission (8.34 Å.) is in agreement with the assignment of the formula $NiO.Fe_2O_3$ (8.34 Å.). No spectrographic analysis is available.

5 CoFe. The spinel occurring in the body of the oxide film cannot be determined uniquely by electron diffraction, since there are two possible spinels, Fe_3O_4 and $CoO.Fe_2O_3$, having almost identical lattice parameters. However, the spectrographic analysis shows the presence of iron, copper, and a trace of aluminum but no cobalt. This would seem to indicate that γ-Fe_2O_3 (8.32 Å.) may be the oxide in the body of the film.

5 MnFe. Transmission data show the presence of a spinel with a lattice parameter of 8.32 Å. Since three spinels are possible Fe_3O_4 (8.40 Å.), γ-Fe_2O_3 (8.32 Å.), and $MnO.Fe_2O_3$, it would appear that γ-Fe_2O_3 is the oxide present in the body of the film. Spectrographic analysis shows the presence of iron, manganese, and traces of chromium and aluminum. Manganese may occur in solid solution in the γ-Fe_2O_3 lattice.

The two remaining alloys, 5 SiFe and 4 WFe, show only the presence of α-Fe_2O_3 by transmission.

The surface oxide on 5 SiFe as determined by the reflection technique appears to be Fe_3O_4. This case is rather unusual, since α-Fe_2O_3 is in contact with the substrate and Fe_3O_4 on the surface. Usually α-Fe_2O_3 is formed only when there is an unrestricted supply of oxygen and equilibrium conditions are being approached.

The surface oxide on 4 WFe appears to be α-Fe_2O_3. This is the only case in this series of alloys where α-Fe_2O_3 is found on the surface.

In summation, the structure of the alloying metal does not seem to have an appreciable effect on the occurrence of a given oxide on the surface. Polishing may have an effect in concentrating the alloying metals in the surface layer of the oxide but this increase in concentration, if it occurs, does not appear to result in the formation of an oxide of the alloying metal on the surface of the film. The data are not conclusive enough to decide whether the alloying metal may or may not be present in the various spinels which occur in the body of the oxide films.

The electron micrographs for these alloys are shown in Figures 9 to 11. The interpretations of these micrographs are given in Table III.

The micrographs of the oxide layers on 5 MnFe, 5 SiFe, 3 VFe, and 4 WFe show the presence of films which are uniform and composed of irregularly shaped crystals with average crystal sizes of 350, 500, 250, and 300 Å., respectively.

The micrographs on 5 CrFe, 5 NiFe, and 5 CoFe show the presence of films which are nonuniform and composed of crystals of irregular shapes with average crystal sizes of 250, 600, and 400 Å., respectively.

There does not appear to be any close correspondence existing between the structure of the alloying metal and the size and shape of the oxide crystals which form when the alloy is oxidized. With the exception of 5 CrFe, all oxidations were carried out under identical conditions. Since the oxidation rates of these alloys are probably different, there may be some differences in the thicknesses of the oxide films.

DEVIATION OF CALCULATED LATTICE PARAMETERS FROM X-RAY VALUES

A previous work by the authors (*8*) indicated that the electron diffraction reflection method gives lattice parameters for the oxide films formed on abraded samples of iron, cobalt, nickel, chromium, and copper which are slightly larger than the x-ray values. These parameters showed negligible time and temperature effects.

Transmission data obtained by the authors (*15*) on the same metals show small negative deviations. These samples had been submitted to metallographic polishing prior to oxidation. On the polished metals reflection data yield lattice parameters which are on the average 0.7% high, while the transmission method gives values which are on the average of 0.2% low.

In a recent paper (*10*) on alloys the lattice parameters of the oxides formed on abraded samples show in general that positive deviations occur. The average deviation was calculated to be 0.3%. The lattice parameter deviations of the oxides found in this work are shown in Table V.

The reflection method yields results which are, on the average, 0.4% high, while the transmission method gives values which are, on the average, 0.3% low. The positive deviations are greatest for Fe_3O_4 and least for α-Fe_2O_3 and Cr_2O_3. The negative deviations result almost entirely for Fe_3O_4 and are negligible for α-Fe_2O_3 and Cr_2O_3.

In the calculation of the deviations from the transmission data, Fe_3O_4 was used as the unknown spinel in all cases. Since other spinels may be present, as discussed earlier, the deviations may actually be smaller than those listed.

The positive deviations for Fe_3O_4 have a range of +0.24 to +0.60% and an average of 0.40%. The α-Fe_2O_3 lattice has an average deviation of +0.35% while the one observation of the Cr_2O_3 lattice gives a deviation of +0.30%. Goldschmidt (*6*) has shown that a variation in the stoichiometric ratio of iron to oxygen atoms in the lattice may give deviations of the order of magnitude observed here. Bernard (*1*) also reports that an increase in the lattice parameter of FeO from 4.28 to 4.298 Å. is caused by the solid solution of iron in the oxide lattice.

The differences observed in the lattice parameters as found by the reflection and transmission methods thus may be due to one or more of the following reasons:

The presence of strains in the oxide film on the alloy and subsequent relief of these strains when the film is stripped.

Transformation of Fe_3O_4 to γ-Fe_2O_3 by the stripping process.

Variations in the stoichiometric ratio of iron to oxygen atoms in the lattice.

Stratification of the several components as a function of the film thickness.

CONCLUSION

Electron Diffraction. The results obtained by the electron diffraction reflection technique show that the oxides found on the outer surface of the oxide layer may not be directly correlated with the composition of the alloy. In some cases oxides are found on the surface by reflection and are not observed after the film is stripped and examined by transmission. The cause of this difference may be a chemical change in the film as a result of the stripping technique or the presence of the outer oxide layer as such a small fraction of the whole film that the transmission technique is not sensitive enough to show its presence.

The difference in composition of the outer layer and the body of the oxide film may result from the following experimental facts observed on the alloys in addition to any possible effects which may result from the stripping technique:

Iron and chromium ions appear to diffuse more readily to the surface than other metal ions.

NiO is never observed on the surface, although it does occur in the body of the film on certain alloys; this may indicate a low diffusion rate for the ion.

CoO is not observed on the surface or in the body of an oxide even when the percentage of cobalt is high as in 30% CoFe; there may, however, be a solid phase reaction between CoO and Fe_2O_3 to form the spinel, $CoO.Fe_2O_3$.

The protective and refractory alloys, such as 13 CrFe, 18-8 stainless steel, K42B, Inconel, and Nichrome V, yield Cr_2O_3 as one of the oxides in the body of the film, although Cr_2O_3 may not be present on the surface of the oxide film. In two of these cases, 13 CrFe and Nichrome V, Cr_2O_3 is found both on the surface and in the body of the oxide film. None of the alloys shows the presence of Cr_2O_3 on the surface if it is not present in the body of the film. This would seem to indicate that in many alloys containing both iron and chromium, Cr_2O_3 is in contact with the metallic substrate while oxides of iron occur in the outer surface of the oxide film. An examination of those alloys shown in Table V which contain both chromium and iron indicates that iron in most cases has a greater tendency than chromium to get to the surface.

All of the magnetic, sealing, and miscellaneous alloys are oxidized in the temperature range 250° to 400° C. On Hipernik the presence of NiO, α-Fe_2O_3, and a spinel with a lattice parameter of 8.34 Å. suggests that a solid phase reaction may have occurred between NiO and α-Fe_2O_3 to form $NiO.Fe_2O_3$ with a lattice parameter of 8.34 Å. On Kovar the spinel in the body of the oxide film may be $NiO.Fe_2O_3$, $CoO.Fe_2O_3$, Fe_3O_4, or a mixture of any two or all three of these, although the lattice parameter of 8.35 Å. suggests that $NiO.Fe_2O_3$ may be predominant. Unfortunately, no spectrographic analysis is available to confirm this. The spinels in the body of the oxide films formed on 5 CrFe, 5 NiFe, and 5 CoFe may be $FeO.Cr_2O_3$, $NiO.Fe_2O_3$, and $CoO.Fe_2O_3$, respectively. Further work on the analysis of these oxide coatings is required in order that more positive identifications may be made.

It is of interest to compare the oxides found on polished samples in this study with those found on abraded samples recently reported by the authors (*10*). These comparisons shown in Table VII are made at the same temperature and time of oxidation although the oxygen pressure was 1 mm. with the abraded and 0.1 atmosphere with the polished samples.

There is a 1 to 1 correspondence of oxides obtained in the two methods of surface preparation. This indicates that polishing appears to have little or no effect or the oxides occurring in the

outer layer of the oxide surface. The oxygen pressure effect also appears to be negligible.

The lattice parameters of the oxides observed on polished samples of the alloys are in general agreement with x-ray values although slightly larger by the reflection technique and slightly smaller by the transmission technique. In those cases where more than one time of oxidation is used, no variation of lattice parameter with film thickness is noted.

Table VII. Comparison of Oxide Film Structures on Abraded and Polished Samples by Reflection Method

Alloy	Abraded Samples	Polished Samples
13 CrFe	α-Fe_2O_3 or Cr_2O_3	Cr_2O
18-8 SS	α-Fe_2O_3 + Fe_3O_4	α-Fe_2O_3 + Fe_3O_4
K42B	Fe_3O_4	Fe_3O_4
Inconel	Fe_3O_4	Fe_3O_4
Nichrome V	Cr_2O_3	Cr_2O_3

The authors' recent study (*10*) indicated that no unique oxide lattice could be correlated with the protective quality of an alloy. Neither could any statement be made relative to the position of the protective part of the oxide film. This study indicates that those chromium alloys, which are protective or refractory, always show the presence of Cr_2O_3 in the body of the oxide layer and may also show it in the surface film. This would seem to indicate that Cr_2O_3 may be functioning as a protective oxide layer and that it is actually in contact with the substrate. Further work is needed where the surface of the oxide in contact with the substrate is examined by the reflection diffraction technique in order to determine if this inner oxide surface actually is Cr_2O_3.

Electron Microscope. The selection of the various temperatures and times of oxidation is made such that the oxide films obtained are of comparable thickness. It is estimated that the thickness range lies between 100 and 300 Å. Within this thickness range all the alloys may be considered to be protective and they are probably obeying a parabolic rate law of oxidation.

The refractory and protective alloys included in this study are all oxidized at 600° C. In cases where two experiments at different times of oxidation are performed, the average crystal size increases with an increase in the time of oxidation. This increase is the most pronounced for stainless steel with relatively slight increases observed for K42B, Inconel, and Nichrome V. Of these four alloys Nichrome V is probably the most protective while stainless steel may be the least protective. The increase in crystal size is greatest for stainless steel and least for Nichrome V. There may, therefore, exist a correlation between the size of the oxide crystals formed and the protective quality of the alloy. Further work involving shorter and longer times of oxidation and higher temperatures is required in order to check this possibility. All the oxide films on these alloys consist of irregular crystals with considerable overlapping. Most of the films are nonuniform.

The magnetic, sealing, and miscellaneous alloys are oxidized in the temperature range 250° to 400° C. All the oxide films are composed of crystals of irregular shapes. The manganese, silicon, vanadium, and tungsten alloys are characterized by uniform films, while the cobalt, chromium, and nickel alloys form nonuniform films. The average crystal size varies more widely on these alloys than it does on the protective or refractory alloys ranging from 250 Å. on 3 VFe or 5 CrFe to 600 Å. on 5 NiFe.

The occurrence of nonuniform oxide films on alloys is not unexpected, since the initial layer may consist of the several oxides which have different rates of growth. In addition, each oxide possesses certain preferred directions of growth. A third factor may be concerned with the ease with which a metallic ion gets to the surface and is oxidized. All these factors would favor the formation of nonuniform films. One might expect, therefore, to find thicker and thinner sections of oxide film existing on the surface. In a previous paper on metals (*15*) the authors have explained overlapping of crystals as the result of the physical overlap of two crystals, and the contact zone between crystals occurring at an angle to the electron beam. This overlapping phenomenon is observed in many of the films on the alloys.

SUMMARY

The thin film oxidation process occurring on alloys composed principally of iron, cobalt, nickel, and chromium is studied by electron diffraction and the electron microscope. Both the reflection and transmission methods of electron diffraction are used. The film stripping techniques developed by Evans and co-workers are used for preparing the film for the transmission studies. Typical commercial protective, refractory, magnetic, and sealing alloys are studied. In addition a miscellaneous group of experimental alloys is included.

The following electron diffraction results are obtained: (1) Iron and chromium ions diffuse more readily to the surface of the oxide film than the other metal ions. (2) Stratification of the oxides occurs even for films of 100 to 300 Å. thick. (3) Cr_2O_3 is always observed in the oxide film on those alloys in this study which are classified as protective or refractory. In those cases where stratification occurs, Cr_2O_3 appears to be in contact with the substrate. (4) NiO is never observed on the surface of the oxide film, although it does occur in certain cases in the body of the film. This fact may indicate a low diffusion rate for the nickel ion. (5) Solid phase reactions may occur between two simple oxides to form the spinel type of oxide structure. (6) With the exception of iron and chromium, metals which constitute no more than 5% of the alloy do not occur as simple oxides on the outer surface of the film. These oxides do appear to form spinels in the body of the film. (7) Simple oxides of cobalt, manganese, silicon, vanadium, and tungsten are not observed in this study.

The following electron microscope results are obtained: (1) The thin oxide films consist of a continuous film of oxide crystals of 100 to 1600 Å. in size. (2) The oxide films are not of uniform density and the crystals have irregular shapes. (3) The effect of longer oxidation periods is to increase the average size of the crystals. (4) The crystals found on the nonprotective alloys such as mild steel at 250° C. are of the same size as the crystals found on the protective and refractory alloys at 600° C. (5) At a given temperature a correlation may exist between the size of the oxide crystals and the protective quality of the oxide film. (6) The thickness of the boundary zone between crystals in the mosaic structure is of the order of 50 Å. or within the resolving power of the microscope.

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Preparation of Powdered Materials for Electron Microscopy

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A discussion is given of the basic problems involved in the preparation of powdered materials for electron microscopy, with special reference to effecting and maintaining an adequate dispersion of ultimate particles. Various tested methods are described for forming thin films of clear resins and for mounting dispersed powders on these films. Procedures are also given for dispersing and mounting powders suspended in resin solutions. The properties and application of various resins and solvents are described, and electron micrographs of several types of mounts are shown. These procedures for mounting particulate materials should be helpful for extension and refinement of the techniques of sample preparation for the electron microscope.

FINELY divided substances were among the first objects studied by the electron microscope, and the literature contains accounts of numerous investigations of specific materials (*9*). In many cases descriptions are given of the methods used to mount the specimens for electron microscopic study, but a general survey of mounting methods for particulate materials has heretofore been lacking. The traditional mounting methods of light microscopy are not in general suitable for the electron microscope, because of the low penetrating power of electrons and the consequent necessity for operating in a high vacuum, which make impossible the use of standard glass slides and immersion liquids. It has therefore been necessary to develop special mounting techniques for electron microscopy. The aim of this paper is to discuss the problems involved in dispersing and mounting powdered materials for electron microscopy, and, since there is no single universally successful method of preparation, to describe various specific procedures found useful in this laboratory.

GENERAL CONSIDERATIONS

DISPERSION. When the purpose of microscopic study is to examine the characteristic size and shape of a few isolated particles, the preparation of a mount can be extremely quick and simple, involving no more than dusting a small quantity of the powder on the thin nitrocellulose films generally used as specimen supports. One purpose of pigment microscopy is to produce micrographs that can be studied statistically to furnish particle-size distribution data (*6*, *12*), from which can be calculated specific surface, covering power of pigments, and other properties. Such statistical study is almost without significance if the particles are clumped together so that few particle diameters can be measured; therefore, the microscopic study must be preceded by adequate dispersion (Figure 1).

An ideally dispersed mount consists of a field of particles crowded enough for many to be studied on one plate, yet with each particle sufficiently separated from its neighbors to permit measuring. The dispersion of a powdered material involves the breaking of aggregates without comminution of the ultimate particles, and the prevention of flocculation during the subsequent preparation of the mount. Some form of work—mechanical, thermal, electrical (*10*), magnetic, or chemical—must be applied in order to break these aggregates down to the ultimate working unit, which is a single crystal, fragment of crystal, or small hard aggregate that functions as an ultimate particle (*4*).

FLOCCULATION. Flocculation is generally considered to occur when a powder is "poorly wetted" by the mounting medium, and if it occurs, the selection of another medium will often bring about a deflocculated mount. If the powder is known to be hydrophobic or hydrophilic, the mounting medium should be chosen accordingly. Wetting agents are sometimes helpful, but there is some evidence that they may make the image of a particle fuzzy by concentrating at the particle-medium interface; therefore, it is preferable to rely on the wetting properties of a properly chosen mounting medium, and to avoid the use of an additional wetting agent.

FILMS AND SCREENS. Electron microscope preparations are usually mounted on very thin (100 to 300 Å.) resin films. The fragile films are themselves supported on 0.3-cm. (0.125-inch) disks of perforated metal (nickel-plated copper), or of calendered screening of stainless steel, phosphor bronze, or copper. The size of hole varies from 20 microns for the electrolytic screens to over 100 microns for the woven screens. The small holes afford more support for films, but the large ones give a greater field of view. It is convenient to have punches of $^1/_{64}$, $^1/_{32}$, and $^1/_{16}$ inch with which to make a large hole in the center of a screen to facilitate the location of large objects in the electron microscope. Such punched screens are also used for electron diffraction work to eliminate all diffracted electrons coming from the surface of the screen wires. The film over such a large hole must be heavier than normally used, but for moderately low magnifications will not interfere with the quality of the image.

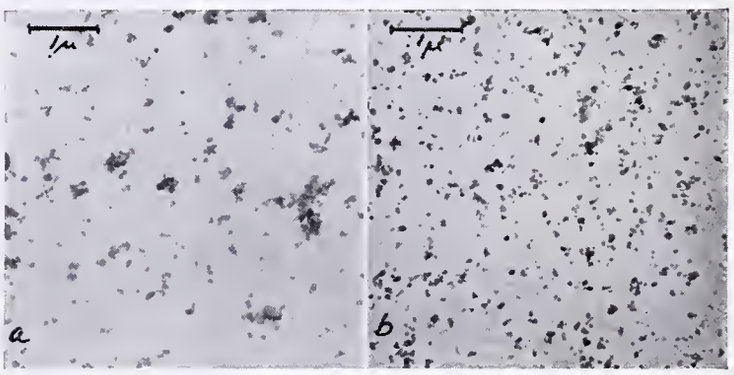

Figure 1. Dispersion

a. Iron blue in nitrocellulose, stirred
b. Iron blue in nitrocellulose, dispersed mechanically

USE OF LIGHT MICROSCOPE. It is strongly recommended that all stages of the preparation of mounts for the electron microscope be followed by observation in the light microscope. Experience in comparative observations with both instruments enables the microscopist to save much time by discarding unsatisfactory mounts before they are ready for the electron microscope.

MOUNTING ON A CLEAR RESIN FILM

FILMS. The resin film should be as nearly transparent to electrons as possible, should show no structure of its own, and should contain no suspended impurities. One exception to this generalization is in the case of stereoscopic studies of large particles, where a little fine dirt on the supporting film supplies a reference plane that is psychologically helpful in studying the stereoscopic micrographs. The thinner the film, the more transparent it will be. An ideally thin film is one which breaks under the full intensity of the electron beam, but which holds together under intensities sufficient to give a clearly visible image on the fluorescent screen of the microscope. A fragile film can be toughened by subjecting it first to very low beam intensities, and gradually building up to the desired brightness, both by bringing the source into focus at the specimen plane and by increasing the beam current. Usually larger particles will require thicker films for proper support.

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Resins. The most generally used resin is a purified nitrocellulose called Parlodion which is soluble in the usual nitrocellulose solvents. Greater toughness and heat resistance are exhibited by a polyvinyl formal polymer, Formvar 15/95, Grade E; this resin dissolves in a relatively limited number of solvents. All the resins listed in Table I, and doubtless many others, may be used to form films, but the two mentioned above have been adequate for a large part of the work in this laboratory.

Spread Films. A common method of film formation is to drop a small amount of resin solution on water, let it spread and dry, place screens on it, and pick up the screens with a special annular tool or with a glass microscope slide. The thickness of the film is controlled by the concentration of the original solution, by the volatility of the solvent (the more volatile solvents evaporate faster, and the film area is consequently smaller than with a less volatile solvent), and by the spreading coefficient of the solution (*1*). The spreading coefficient is a function of the surface tension of the water, the surface tension of the solution, and the interfacial tension of the two liquids; the coefficient must be positive if spreading is to occur. In the absence of a surface-active agent, the spreading coefficient of the solvent controls the spreading of the solution. Thus, Parlodion in methyl, ethyl, amyl, or Cellosolve acetates (all with positive coefficients) spreads well; whereas Formvar in ethylene dichloride or dibromide (negative coefficients) will not spread, but forms lenses on the water instead. Formvar (5%) in dichloroethylene or propylene dichloride will spread fairly well, since the unsymmetrical nature of those molecules enables them to spread. Formvar in ethylene dichloride will spread if it contains a sufficient amount of wetting agent, such as Aerosol OT (suggested by A. Y. Mottlau, Standard Oil Development Co., Elizabeth, N. J.) or oleic acid, although the films obtained with the former contain many holes and are lacy in appearance. This lacy structure may be desirable as a support for weak or exceptionally thin films, and can also be achieved by dispersing camphor in an equal or slightly larger volume of 10% Parlodion in amyl acetate and spreading the mixture on water.

The water on which the films are spread is contained in a rectangular dish of Pyrex whose edges are ground flat, and whose inner surface and edges are lightly coated with paraffin. When the dish is filled to the brim with distilled water, the meniscus rises above the edge of the dish and can be swept clean with paraffined glass rods or chromium-plated brass bars. The water is swept twice in this manner before every operation. The distilled water must be changed daily, since any impurity tending to lower the surface tension of the water will concentrate at the water-air interface and seriously reduce the spreading of solutions on the water. Any bacteria growing in the water will be observed on the preparation; therefore the dish must be cleaned regularly.

Mercury may also be used as a substrate on which to spread films. Its high surface tension makes it theoretically excellent for this purpose, but the practical difficulties of cleaning and handling mercury are considerable.

Cast Films. Resin films can be formed by casting onto glass. Dilute solutions of the resin (0.2 to 0.4% by weight) in very volatile solvents are taken up in a micropipet formed by drawing out soft glass tubing in a flame (*2*). A slight bend in the tubing will prevent the solution from running back into the rubber bulb and becoming contaminated. In forming the film, the long narrow shank of the pipet is held close to and parallel with a clean glass slide; the liquid is gently expelled and is drawn between pipet and slide by capillarity, then quickly spread out across the slide with a sideways motion of the pipet. To eliminate streaking, the slide is held vertically in an atmosphere of the solvent for the resin, preferably in a large jar or beaker, where it is allowed to drain until dry. The film thickness depends on the concentration of the solution, the volatility of the solvent, the length of time for draining, and to some extent on the manner in which the solution is spread. The film can be formed more simply by dipping the slide into a jar of the solution, but the pipet method is preferred for two reasons: the large surface of the dipping jar exposes the solution to dust contamination, an important consideration in many laboratories, and the tendency of ethylene dichloride (the usual Formvar solvent) to become acid on exposure to light and air is aggravated. All ethylene dichloride solutions should be stored in brown bottles, since a markedly acid solution forms weak films that are difficult or impossible to strip.

Manipulation of Cast Films. After casting and drying, th film is scored around the edge with a needle and usually cut int 0.25-inch squares for easy manipulation of the screens. Th slide is lowered into the water at a low angle, film side uppermost and the edge of the film is teased loose from the slide with a needle This is easier if the rim is thickened with one or two extra coats o resin. The film slowly peels off the slide and floats on the surfac of the water with little help from the needle; breathing on th slide before and during peeling will facilitate the operation. I the squares of film show any interference colors when examine by reflected light, they are too thick. A pale, first-order gray i the color of the heaviest film that can be employed; the best film are nearly invisible on the water surface.

The glass slides should be clean and free from scratches, be cause scratches are reproduced in the film. Dirt will contaminat the film and sometimes prevent it from peeling off the slide To minimize these difficulties, new microscope slides of goo quality are employed; they are cleaned in soap and water, rinsed and dried. Just before use, a slide is removed from the water drained, then polished with fine magnesium oxide on a small wad of moist lens paper, and wiped dry with a clean soft cloth. If the slides are used only for this purpose, they may be cleaned and reused several times before becoming too badly scratched.

The film may be transferred directly from the slide to the screen by a method described by Schaefer and Harker (*13*). The screen is placed on a film-covered slide, both are covered with condensed moisture by breathing on the slide, and Scotch tape is quickly pressed down over screen and slide. When the tape is gently peeled off the slide, the screen comes up with the film on it.

Dry Mounting. The specimen may be placed on the film in a variety of ways. The simplest is to dust the dried powder onto either a film-covered screen or a film-covered slide. In the latter case, a suitable area is selected in the light microscope, and a screen is placed over that area. A small drop of rubber cement on one edge of the screen will keep it from slipping off the selected area as film and screen are peeled off together.

Mounting Powders Suspended in a Liquid. Any powder that can be dispersed in a volatile liquid which does not dissolve the film on the screen, can be easily mounted by placing a drop of the suspension on a film-covered screen and allowing it to dry; however, the particles tend to clump together as they go to dryness. It is possible to keep the particles dispersed to the last moment by applying some form of vibration, as described below. It is also possible to immobilize a deflocculated suspension by quick-freezing in liquid air or a dry ice–alcohol mixture, and then to sublime the ice under high vacuum. Particles can also be directly centrifuged onto the film-covered screen (*3*).

Removing Soluble Impurities. Soluble impurities in a suspension are often troublesome, since they crystallize on drying and are visible in the electron microscope. The mounting film can be used as a dialyzing membrane to remove water-soluble substances: a drop of the suspension is placed on the film as it lies on the water and water is allowed to dialyze through the film. The drop swells as water passes through the film by osmosis. A screen is placed on the drop, and the excess water withdrawn by

Table I. Resins and Solvents

Resin	Recommended Solvents: For dispersion[a]	For mounts cast on glass[b]	For mounts spread on water[c]	For mounts drawn down on glass[c]
Nitrocellulose (Parlodion)	Cellosolve acetate	Methyl acetate Ethyl acetate	Methyl acetate Ethyl acetate Amyl acetate Cellosolve acetate Octyl acetate	Ethyl acetate Amyl acetate
Formvar 15/95	Dioxane plus a few drops of dimethyl dioxane Nitroethane	Ethylene dichloride Nitroethane	Dichloroethylene Propylene dichloride Ethylene dichloride plus 0.1% oleic acid Nitroethane 1,1-Dichloro-2-nitroethane	Dioxane Nitroethane
Polystyrene	Xylene	Ethylene dichloride Toluene	Benzene Toluene Xylene	Benzene Toluene
Methyl methacrylate or cellulose acetate	Methyl amyl ketone Methyl ethyl ketone plus 10% isophorone	Acetone	Methyl ethyl ketone Methyl amyl ketone Isophorone	Methyl ethyl ketone

[a] 10 to 20% solutions, approximately.
[b] 0.1 to 0.5% solutions, approximately.
[c] No fixed concentration; suspension allowed to dry to suitable consistency.

micropipet, dropping the screen on the film. Film and screen re removed and dried in the usual manner.

DISPERSION OF POWDERS

Turpentine Dispersion. The manner in which work is applied to obtain a dispersion may vary greatly. A standard pigment-dispersion method used in light microscopy may be adapted or electron microscopy (*5*).

The pigment is rubbed out on a microscope slide in a drop of esh triple-distilled turpentine, using a back and forth circular otion of a glass rod held parallel to and touching the slide horiontally. As the last of the turpentine evaporates, the particles y in wedges. In some areas of these wedges, the particle denty will be an optimum, neither too crowded nor too sparse. Such dispersion may be flooded with a dilute resin solution, drained, ied, and stripped onto water, as in the formation of a cast resin m; or it may be stripped by the method of Schaefer and arker. The film partly surrounds the pigment particles and ts them off the slide.

This method works fairly well with powders of large and fairly iform particle size (0.5 micron and greater). The method may so be reversed—that is, the turpentine dispersion may be made rectly on a film-covered slide. The film is unavoidably torn by e largest particles under the rod, and it may be awkward to el onto water; however, a continuous scrap of film $^1/_{16}$ inch uare is ample for electron microscopy.

Dispersion of Soft Substances. Many substances are too ft to be rubbed out mechanically without deformation. These aterials can be placed in a mortar with solid carbon dioxide and ound until just before the dry ice has all sublimed. The low mperature makes most soft substances sufficiently brittle to atter. The particles can later be mounted in any convenient anner. Since commercial dry ice contains appreciable amounts oil, the particles should be washed by decantation with an oil lvent.

Dispersion with Vibrators. Air flocculates may sometimes broken apart using a simple mechanical vibrator. The dry mple is placed on a film-covered microscope slide fastened to the brator. Materials of very large particle sizes have a tendency to parate into different size ranges as they are vibrated. Sonic bration in the upper audio range (15,000 cycles per second) as also tried. An arm rigidly attached to the vibrator of a loudeaker strikes the bottom of a glass cell made of a ground-glass ng cemented to a cover slip. The cell contains water on which floated a film-covered screen, which in turn supports the drop liquid containing the sample in suspension. Supersonic vibran (550 kilocycles per second) was used in a similar manner (*7*). watch glass containing water is placed directly on an electrode the supersonic vibrator and a screen floated on the water as bere. The latter two methods were tried only with carbon black spended in water. Although the results were not entirely tisfactory, the method as described might be useful in special oblems, upon proper selection of suspending medium and adstment of particle concentration.

Dispersion with a Spark. Dispersions may also be made usg a high-frequency spark generated by a Tesla coil. The dry mple is placed in a small pile on one end of a film-covered microope slide, and the slide is clamped on a wooden support with e sample-bearing end projecting. A metal plate is supported out 0.25 inch above the slide. A spark from the coil is passed veral times across the bottom of the slide. The powder scatters dially outward and usable areas may be found around the edges the slide. The spark cannot be applied directly to the sample, nce it alters the film, making it difficult to strip onto water. his method gives fair results, but is unsuitable for easily fused aterials.

Dispersions with Mills. Mechanical dispersions may be ade in ball, colloid, rubber, or roller mills, or any others, but these commercial mills require a relatively large sample, consume considerable time, and in some cases contaminate the sample severely. It should be remembered that nitrocellulose mixtures may degrade or explode on milling. Mortar and pestle, muller and plate, or an all-glass motor-driven micromill (*11*) are cleaner and use a smaller sample, but they have not been found to give the most satisfactory dispersions. An agate mortar and pestle are indispensable for very refractory materials.

Dispersion with a Spatula. The authors have found that the best dispersions for electron microscopy are made with the edge of a stiff spatula on a flat white glass table with a fine-grain "suede" finish. The table is cleaned and re-surfaced with No. 600 Carborundum, followed by a good cleansing powder, and liberal rinsing. Plate glass surfaced in the same manner is satisfactory. The best spatulas are of the straight palette knife type, with a fairly stiff 4-inch blade; the edge should be dull, smooth, and straight, and the blade kept polished brightly with fine emery paper. The use of stainless steel is advantageous, as some of the solvents may corrode an ordinary steel blade. Only the largest aggregates are comminuted by being pressed directly against the table by the knife, since fine particles are usually smaller by many orders of magnitude than the average distance between the relatively rough dispersing surfaces. Dispersion is largely accomplished by creating shearing forces in the liquid in which the particles are suspended; the greater the viscosity of that liquid, the greater will be the shearing force that can be produced. This desirable condition is attained by mixing the powder with a solution of the mounting resin in a solvent of moderate volatility. As the solvent slowly evaporates, the mixture becomes more and more viscous. Just before going to dryness, it has the consistency of tar and can be smeared out on the dispersion table.

The knife is held with the edge parallel to the table and the blade tilted slightly in the direction of smearing, which is normal to the edge of the knife. The mixture is rubbed out over an area of a few square inches, scraped up, and resmeared until it is too stiff to work without breaking the spatula. The mixture may be wetted with more of the same solvent and reworked, if the dispersion is not complete. The solvent should have sufficiently low volatility to give adequate rubbing time, usually about half an hour for 0.5 cc. of mixture. After the initial mixing, it is not necessary to rub the sample continuously, since the most effective work is accomplished in the last 10 minutes, and several dispersions can thus be in progress at the same time.

Particle Density. The proportion of dry pigment to resin solution that will give a mount of proper particle concentration varies with the average particle size of the pigment. The finer the pigment, the smaller is the proportion of pigment volume to resin-solution volume, since, of course, a given weight of a fine pigment has a greater projected area than an equal weight of coarser pigment, and consequently will produce a more crowded field. Thus, 1 or 2 mg. of a fine carbon black will be adequate for 0.5 cc. of 10% resin solution, while a bulk of a coarse lead white equal to or greater than that of the resin solution will be needed to produce a sufficiently concentrated mount.

Mounts for Electron Diffraction. Pigment mounts for electron diffraction must be more dense than for microscopic studies, in order to provide a sufficient number of surfaces for diffraction, so that continuous rings rather than separate spots will be formed on the photographic plate. It is possible to obtain fairly satisfactory micrographs and diffraction patterns from the same preparation, by making a compromise in the particle density between optimum density for diffraction and optimum density for micrography.

MOUNTING DISPERSED POWDERS

After a powder has been dispersed in a resin, there are several methods for forming it into a film suitable for electron microscopy. Each method has its advantages and disadvantages; consc-

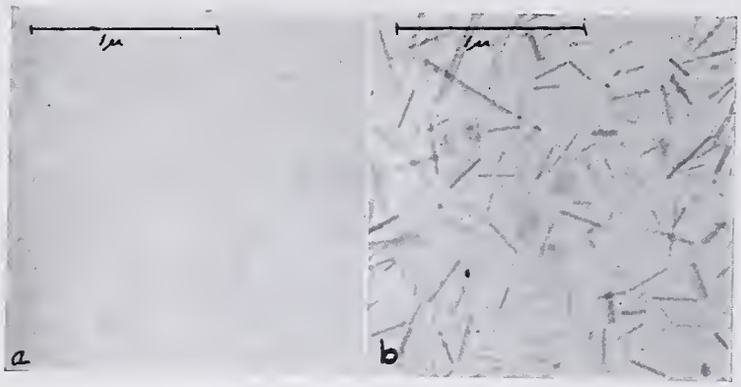

Figure 2. Mounts Cast on Glass

a. Phthalocyanine green in Formvar
b. Attapulgus clay in Formvar

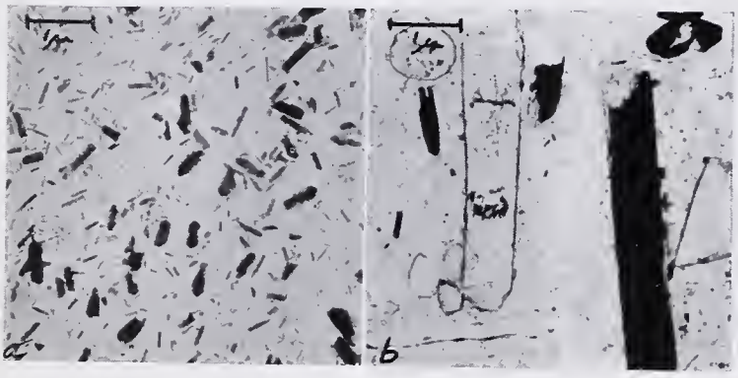

Figure 3. Mounts Spread on Water

a. Iron oxide in nitrocellulose
b. Antimony oxide in nitrocellulose; although this material volatilizes in the electron beam, the particle size and shape are clearly outlined by the nitrocellulose

quently, the choice of a mounting method must be made with reference to the particular substance to be studied.

Cast Mounts. Materials of colloidal dimensions dispersed in a resin may be resuspended in a fairly volatile solvent and cast onto a clean glass slide as described above. The dry smear on the dispersion table is taken up with solvent and transferred to a test tube. A small amount of this is placed in another test tube and diluted with the same solvent. From this suspension a film is cast, and if it is not thin enough, the dilution is repeated and another film formed. This procedure is continued until the film becomes too thin to remain coherent when peeled onto the water; the dilution just preceding this one is used to make the final film.

While this method is tedious, it produces films of exceptional thinness and clarity, and is the only method suited to the finest carbon blacks and organic pigments. Formvar diluted with ethylene dichloride is usually employed because of its superior toughness. Materials that flocculate while being cast cannot be mounted in this way, but another combination of resin and solvent may sometimes avoid flocculation. Materials that have too large a particle size to stay in suspension for several minutes cannot be mounted by casting, since the larger sizes will settle out in the test tube, and the mount will not properly represent the original sample. Figure 2 shows mounts typical of the casting method and Table I suggests various resin and solvent combinations.

Spread Mounts. A method modified from one originally suggested in the RCA electron microscope instruction book is useful for medium-sized particles (0.2 to 2 microns). For this method the dispersed mixture is taken up in a sol ent of positive spreading coefficient, allowed to evaporate until it reaches the consistency of thin cream, and then spread on water by drawing a drop on the end of a spatula rapidly across the surface. Screens are placed on thin, carefully chosen areas of the spread film, removed, and dried as usual (Figure 3).

This method is quick and convenient, but has the disadva tage that the films often contain many holes (Figure 4, *a*), f reasons not clearly understood. While electrostatic charges the electron microscope have been blamed for this condition, tl holes are often visible in the light microscope when the film is st moist and before it is placed in the electron microscope. Res solutions containing surface-active agents may form lacy film suggesting surface activity as one cause of hole formation. The is probably also some connection between hole formation ar water-solubility, since holes are readily formed in nitrocellulo films when using the highly water-soluble esters, methyl forma and methyl acetate, and also with the highly insoluble octyl ac tate. Solvents of moderate water solubility seem best for sprea ing nitrocellulose, as, for example, amyl acetate and Cellosol acetate. In the case of resins such as Formvar and polystyren which dissolve only in almost totally water-insoluble solvent the relative water solubility of the solvents seems to have no influ ence on hold formation. The holes in the film are sometime found concentrated around the edges of the particles (Figure 4, *a* indicating poor wetting of the particle by the solvent, but mor frequently the holes have no apparent connection with the pres ence of particles.

There are other variables affecting the quality of spread films such as the concentration ratio of pigment to resin to solvent, th cleanliness and temperature of the water, the speed with whic the knife carrying the suspension is drawn across the surface o the water, and most important, the choice of area on which t place a screen, since spread films are very nonhomogeneous. Th procedure is highly empirical, but with a little practice consist ently good results can be obtained with any powder to which th method is suited.

"Draw-Down" Mounts. The most widely applicabl method of mounting a dispersed powder is to make a draw-dow on a clean glass slide, strip the dried film onto water, and plac screens on the thinnest areas (Figure 5, *a*, *b*, *c*, *d*). In this metho the dispersed mixture is suspended in a solvent of volatility equa to or greater than that used for spreading films on water; it is then stirred and allowed to evaporate to the consistency o heavy cream; the mixture is finally drawn down on a clean glass slide in a series of short strokes, using either the edge or the tip o the spatula. After drying, the film is cut into squares and floated off onto water.

Such a film exhibits a great variation in thickness, and the screens are placed on the thinnest areas. Even the thinnest areas consist of microscopic stripes of different thicknesses resulting from the irregularities of the knife edge. When such a film is examined in the electron microscope, some screen holes are seen to have no film, and others will be only partially covered, but many will be completely covered with film. The irregularities of the mount assure a large range of particle density on any one specimen screen, so that a field of any desired density may be found upon sufficient scanning. This type of mount should be made with a somewhat higher pigment-resin ratio than the other methods. Samples mounted in this manner exhibit fewer holes and less flocculation than other methods of mounting. If there is some tendency to flocculation, it may be minimized by employing a more volatile solvent and making the draw-down more rapidly. By this means, the particles are mechanically deflocculated and fixed in position by drying before they have time to reflocculate. This method is excellent for particles of all sizes except the extremes of fine and coarse.

Draw-Downs in Liquids. Powders of the largest particle size range for which the electron microscope is useful (1 to 10 microns) are mounted by a variation on the draw-down procedure. The powder is wetted with a minimum amount of a high-boiling hydrocarbon (such as ink oil No. 896, Gulf Refining Co., boiling point, 270–315° C.; this oil must be redistilled under vacuum and stored in a brown bottle) or dibutyl phthalate and dispersed on the table. It is then drawn down on a film-covered slide with

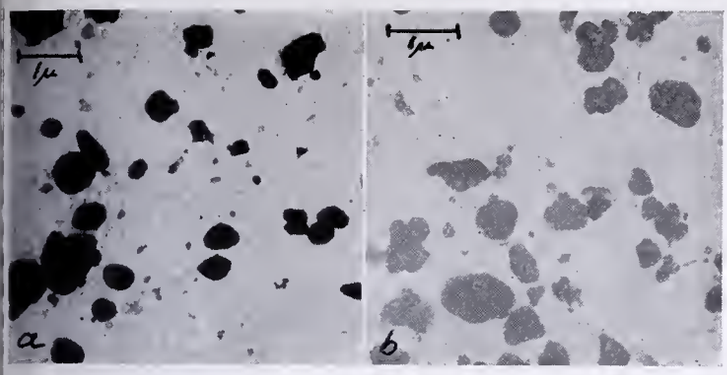

Figure 4. Holes in Films Near Particles

a. Chromium oxide in nitrocellulose, spread on water
b. Chromium oxide in nitrocellulose, drawn down on glass

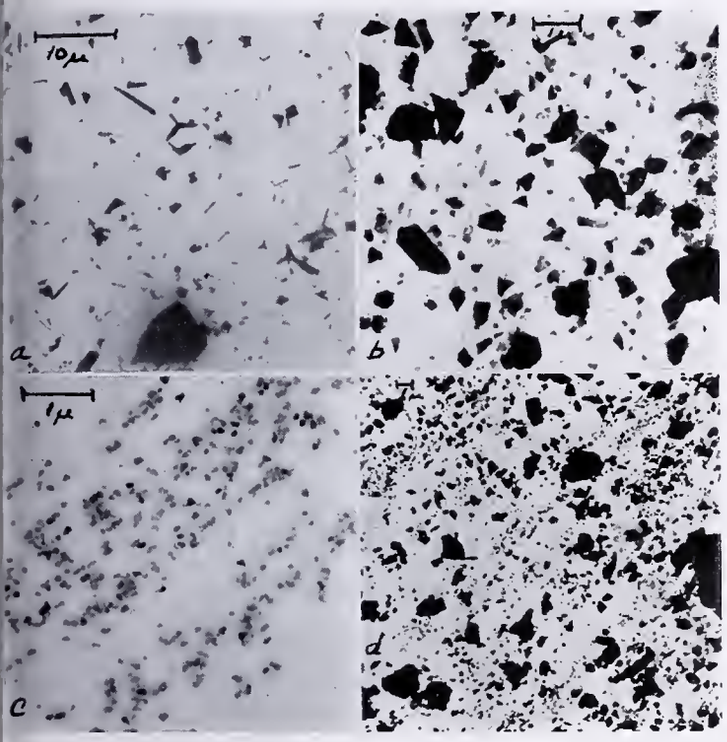

Figure 5. Mounts Drawn Down on Glass

a. Zinc oxide in Formvar
b. Ultramarine in nitrocellulose
c. Iron blue in castor oil
d. Calcium carbonate in a high boiling hydrocarbon

hort strokes as in the resin draw-down method. The film is loated onto water and mounted on screens with large holes. The hydrocarbon and dibutyl phthalate are practically nonvolaile at atmospheric pressures and room temperatures, but disppear completely in the high vacuum of the electron microscope. The dispersions made by this method are never so complete as n the resin draw-down method, since the lower viscosity of the ydrocarbon limits the shearing action that breaks the aggreates. It is, however, possible to study large particles stereocopically to aid in the differentiation between an aggregate and single particle. Since the particles are not incorporated in the ilm, they seldom rupture it under electron bombardment (Figure , *d*).

Suspensions of solids in semivolatile and nonvolatile media, uch as paints or printing inks, may be mounted for electron miroscopy by smearing them onto film-covered slides (*8*). The nonvolatile residues naturally cause the electron beam to scatter, with the result that the images of the particles are rather fuzzy (Figure 5, *c*). There is, however, an obvious advantage in studying such material in a condition as near as possible to its original tate.

The various methods of mounting powders can be modified and combined to suit different materials. For instance, a powder in a slurry may be drawn down on a glass slide, dried, and thereafter handled in the manner of the turpentine dispersions. Different conditions will suggest different modifications of mounting methods, with consequent flexibility of application of the electron microscope to the field of powdered substances.

ACKNOWLEDGMENT

The authors express their thanks to Interchemical Corporation for permission to publish this paper; to Henry Green for his valuable advice; and to Vincent Salines, Olive Hodgson, and Gertrude Pfeifer for their able assistance

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Calculation of Specific Dispersion of Pure Hydrocarbons and Petroleum Fractions—Correction

Above is Figure 1 as it should have appeared in connection with the article by M. R. Lipkin and C. C. Martin entitled "Calculation of Specific Dispersion of Pure Hydrocarbons and

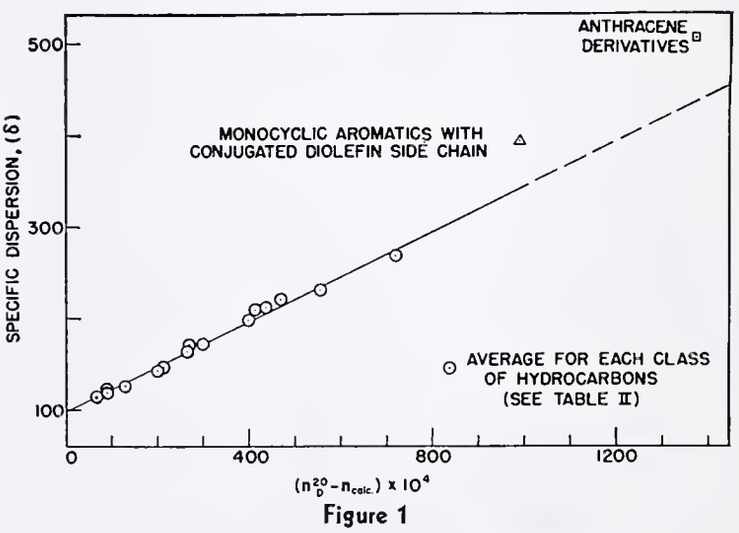

Figure 1

Petroleum Fractions" [Ind. Eng. Chem., Anal. Ed., 18, 433 (1946)]. As will be seen, the point for anthracene derivatives on the graph agrees with the numerical data for this point as given in Table I, page 433, of the article. This was not true in the figure published with the article, owing to an unintentional alteration in the process of preparing the engraving.

The Editors

NOTE ON ANALYTICAL PROCEDURES

Analysis of Boron Trifluoride in Organic Liquids (Ethers)

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CONSIDERABLE difficulty is encountered in analyzing boron trifluoride in organic liquids by conventional methods for boron and fluorine. This is due in part to the fact that the hydrolysis of boron trifluoride gives not only a mixture of boric and hydrofluoric acids, but some fluoboric acid, HBF_4, in addition. Several methods of decomposition have been used. Pflaum and Wenzke (*2*) first made a fusion in a Parr sulfur bomb and determined boron and fluorine in the products of fusion. Bowlus and Nieuwland (*1*) heated with fuming nitric acid in a closed tube to decompose the sample. Vaughn and Nieuwland (*3*) determined fluorine in organic compounds by the use of liquid ammonia and sodium, but did not report any results for boron.

A problem in this laboratory required the routine determination of boron trifluoride dissolved in various low-boiling ethers. These solutions contained no nonvolatile matter. A convenient method of analysis was developed based on the reaction of boron trifluoride with sodium fluoride to form sodium fluoborate. This compound is stable and can be heated to 200° to 300° C. with no appreciable decomposition; therefore, the method should apply to organic solvents with rather high boiling points.

PROCEDURE

About 5 grams of anhydrous sodium fluoride were placed in a 100-ml. wide-mouthed extraction flask, and the flask was stoppered and weighed. Approximately 2 grams of the sample were placed in a tared 15 × 40 mm. weighing bottle and weighed. Samples were conveniently transferred with a small hypodermic syringe and in a dry-box, since boron trifluoride compounds are very hygroscopic.

The weighing bottle and sample were placed in the flask with tweezers, the stopper was removed, and the flask was attached to a reflux condenser. After refluxing for about 30 minutes, the flask was removed and the liquid evaporated on a hot plate. The flask was stoppered, cooled, and weighed. The gain in weight minus the tare of the weighing bottle gave the weight of boron trifluoride in the sample.

Analysis by this method was found to be reliable and accurate within 0.5%.

Three analyses on a single sample of boron trifluoride ethyl etherate gave 48.3, 48.1, and 48.5% boron trifluoride; the theoretical boron trifluoride content was 47.8%. Additional work on mixed butyl and ethyl etherates indicated satisfactory results with this method.

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CORRESPONDENCE

Effect of Acetic Acid on the Spectrophotometric Estimation of Gossypol in Aqueous Alcoholic Solution

SIR: Since publication of an improved rapid method for the determination of gossypol (*1*) it has been found with different lots of alcohol that the intensity of the color and the precision of the analyses depend upon the acidity of the alcohol used.

Very satisfactory results are obtained when sufficient acetic acid (0.1 to 0.2 ml. per liter) is present to give the 95% alcohol an acidity of 0.002 to 0.004 N. The upper limit of acidity is not critical, since as much as 6 ml. of glacial acetic acid per liter may be used.

Table I. Effect of Acetic Acid on Development of Color of Dianilino Gossypol in Aqueous Alcohol

[As measured by per cent transmittance[a] at 445 mμ (6 determinations each concentration)]

	Per Cent Transmittance					
	0.025 Mg. of Gossypol per 25 Ml.		0.100 Mg. of Gossypol per 25 Ml.		0.175 Mg. of Gossypol per 25 Ml.	
	No AcOH	0.2 Ml. of AcOH per liter	No AcOH	0.2 Ml. of AcOH per liter	No AcOH	0.2 Ml. of AcOH per liter
Lowest	77.4	74.0	35.7	30.4	15.6	12.4
Highest	92.0	76.4	47.5	31.6	24.5	13.0
Mean	82.38	74.96	41.03	30.93	22.32	12.96

[a] Coleman double monochrometer spectrophotometer.

Table I shows that the addition of only 0.2 ml. of glacial acetic acid per liter of alcohol increases the intensity of the color and greatly reduces the variation between replicate determinations. The acid added increased the normality from 0.0003 to 0.0037. The equation for the concentration–log transmittance curve for all data with acidified alcohol is: mg. of gossypol in 25 ml. $= \frac{2 - \log T}{5.017}$. This equation agrees with that previously found and published.

It is recommended that in the determination of gossypol by the previously published method (*1*) the alcohol used for the extraction be adjusted to an acidity between 0.002 and 0.004 N with acetic acid.

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Determination of Extinction Corrections in Infrared Analysis of Gaseous Hydrocarbon Mixtures

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Infrared analysis of gaseous hydrocarbon mixtures involves determination of extinctions for the individual components. Experimental data must be corrected for second-order effects due to energy scattered within the instrument, the structure of the absorption band, and intermolecular action. A simple relationship permits correction for these deviations. The method simplifies the work connected with the calibration or recalibration of a spectrophotometer. Use of a reference substance is suggested as a diluting agent in cases where molecular interaction influences absorption.

INFRARED absorption spectrophotometry is slowly acquiring an important position in the routine testing of light hydrocarbon mixtures. Speed is one of the important requirements of these methods, and because much time may be lost in computing the results of infrared analysis, development of abbreviated procedures for such calculations becomes of considerable importance.

In the infrared analysis a certain proportion of time is spent in estimating corrections for second-order effects, the magnitude of which varies with the pressure and composition of the hydrocarbon mixtures. The normal procedure involves the use of several correction factors, but their separate determination and practical application are somewhat tedious because of the absence of a straight-line relationship between the factors involved (*1–6*). The present paper describes a simple method for developing such relationships which has been in successful use for a number of years in these laboratories.

Infrared analysis is based on the law of Beer and Lambert, which is applicable to monochromatic radiation as absorbed by an ideal absorbing medium. The law states that for equal intensities of transmitted light the concentration of the absorbing matter is inversely proportional to the thickness of the absorbent layer. On this basis the following expression gives the relationship between the number of molecules, n, distributed along the path of the radiation and the extinction, E:

$$n = kE \tag{1}$$

where k is a constant. The extinction is obtained as the logarithm of the ratio of the incident to the transmitted radiation intensities. Radiant energy absorption expressed in this form is useful in infrared analysis because of the near approach in actual practice to the relationship expressed in Equation 1. However, it is frequently necessary to consider a small correction term to bring about linearity.

The extinction contributions of individual absorbing substances are considered numerically additive, subject to certain conditions which are taken into account below. In analyzing mixtures attempts are made to select some specific wave length for each individual component at which interference from the absorption bands of other components present will be negligible. This seldom can be achieved in practice and, as a result, a system of simultaneous equations must be solved in order to establish the effect produced on the observed extinction by the extinction contributions from each of the individual constituents of the mixture, so that they may be subtracted in effect. Either before proceeding with or during the course of these calculations the values of the observed extinctions must be corrected for the following sources of error in their measurements:

1. Correction for the energy scattered within the instrument and reaching the recording device by a circuitous path. This correction is regarded as an instrumental factor. Various methods are in use for making this correction, such as subtracting a small numerical value from the energy readings (*1, 3, 4*).
2. Correction for the energy received by the recording device from bands other than the one under investigation. This correction is for that energy portion of the optical beam which is not materially affected by the sample. It is exhibited in the case of an absorption band of the narrow or line type of structure and is the so-called error of band shape and finite slit widths (*3, 5*). The emphasis here on the energy consideration is that from the energy viewpoint this source of error can be treated in a manner similar to that in the case of the first type of error.
3. Correction necessitated by the unique absorption response exhibited by certain molecules in the presence of other molecules. This effect comes under the heading of pressure broadening of a band (*5*).

The proposed correction method outlined below makes it clear that carefully planned auxiliary experiments will be required to distinguish and identify the type of effect being corrected, since the effects themselves are of dissimilar origin and are yet corrected in a similar manner. For simplicity all these corrections may be regarded as a single correction for a particular curve. This can be considered as part of the simplification introduced by the present method.

By plotting observed extinction, E_o, for an individual hydrocarbon against partial pressure a curve is obtained. A difference, ΔE, will be exhibited between the observed extinction and the theoretical extinction. This is demonstrated in Figure 1.

Inspection of a large number of curves of the above type shows that for a particular curve:

1. Deviations from the theoretical tangent increase with increase in magnitude of the observed extinction.
2. The magnitude of the deviation or correction depends on the magnitude of the extinction and is independent of the nature and the pressure of the other hydrocarbons present, subject to the establishment of certain experimental conditions.
3. Corrections to be applied to the observed extinctions are proportional to the square of these values. Mathematically this relationship may be expressed as follows:

$$E_c = E_o\,(1 + \alpha E_o) \tag{2}$$

where E_c is the corrected extinction.

The numerical value of the coefficient, α, varies with the instrument and instrument setting and should be determined experimentally as described further below.

The simplifications developed in this work involve the direct application of corrections to the observed extinctions. Usually only one curve at a given wave length requires correction. However, in a case where two curves at the same wave length require corrections an approximate correction can be assigned, and after the calculations are carried out, the exact correction may be estimated and applied.

Examples of bands which may be selected for routine inspection of hydrocarbon mixtures and which follow the above rules are shown in Table I. This tabulation is by no means exhaustive with respect to the hydrocarbons enumerated or to other absorption bands associated with the individual hydrocarbons listed.

Practical application of the suggested correction method for the observed extinction values may be visualized from the following detailed description of the individual steps in calibrating or recalibrating an instrument.

1. Determine the extinctions at various partial pressures for absorption bands, each of which shows the largest possible deviation from a straight line at the instrument settings to be used. The isobutane band of the approximate wave length 8.4 microns is a suitable example for this purpose. Plot these extinctions against partial pressures reduced to the reference temperature and corrected for differences in deviation from the ideal gas laws (Figure 1).
2. Draw a tangent to the above curve passing through the origin. This tangent represents the theoretical values of the corresponding observed extinctions and the corrected extinctions computed by the use of Equation 2 should fall closely along this tangent.
3. Determine the difference between the theoretical and the observed extinctions at some suitable arbitrary pressure. In order to minimize the error in further calculations the largest difference which corresponds to the highest partial pressure should be selected.

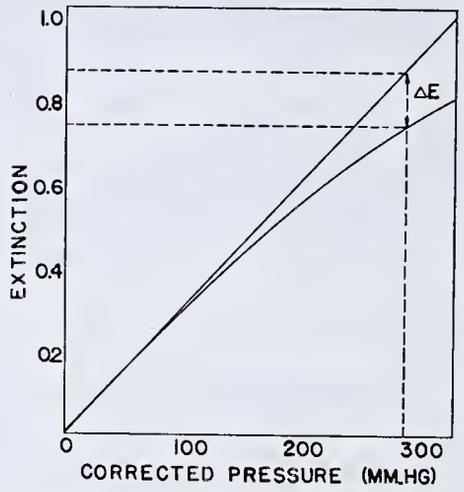

Figure 1. Deviation of Observed and Theoretical Extinctions

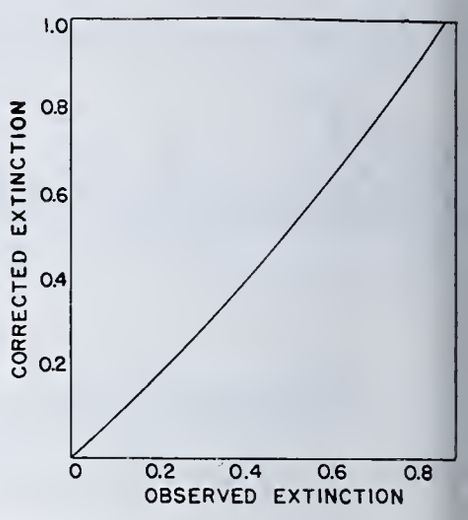

Figure 2. Relation of Corrected Extinction to Observed Extinction

As it follows from further description, establishment of the complete curve for observed extinctions at varying pressures is necessary only for locating the position of the tangent. After this position is established one point on the observed extinction curve is sufficient to locate the remainder of the curve. If this method is used, selection of the point and its very exact location become of primary importance.

4. Determine the value of the coefficient, α, in Equation 2 from the data obtained under item 3. Using this value, plot the corrected extinctions against the observed extinctions by employing this equation and substituting different values for E_o (Figure 2). In some instances a plot of observed extinctions against the correction factors is advantageous.

By assuming the validity of Equation 2, a mathematical method may be readily developed by which α may be determined directly from the experimental data.

As a further illustration, the following example is given of a practical application of the developed relationships.

Assume that an extinction corresponding to a certain band representing a hydrocarbon in a mixture under analysis has been determined as E_o, and the extinction as determined for the pure hydrocarbon is E_{op}. Referring to Figure 2, the corresponding corrected extinctions would be E_c and E_{cp}. Therefore, the per cent hydrocarbon in the mixture is equal to

$$\frac{E_c}{E_{cp}} \times 100$$

In the above example no corrections are shown regarding the interferences in the absorption band that may be caused by other hydrocarbons present. These corrections are made by solving a series of simultaneous equations as described in standard manuals.

The accuracy of the described correction method may be visualized from the following typical examples.

Table I. Absorption Bands of Hydrocarbons Suitable for Use with Proposed Correction Method

Hydrocarbon	Approximate Wave Length, Microns
Methane	7.6
Ethylene	5.2
Ethane	12.0
Propane	13.2
Isobutane	8.4
Butene-1	5.5
Butadiene-1,3	6.3
cis-Butene-2	10.2
n-Butane	10.2
Isopentane	10.2
2-Methylbutene-1	11.0
Dimethylbutane-2,3	8.7

Table II. Comparison of Observed, Corrected, and Theoretical Values of Extinctions for *n*-Pentane

(Wave length 13.7 microns, various pressures. Corrections for deviations attributed to scattered energy)

Partial Pressure, Mm. Hg	Extinction			Deviation from Theoretical, %	
	Observed	Corrected	Theoretical	Observed	Corrected
50	0.076	0.077	0.076	0.0	1.3
100	0.151	0.154	0.152	0.7	1.3
150	0.222	0.228	0.228	2.6	0.0
200	0.293	0.303	0.304	3.6	0.3
250	0.363	0.379	0.380	4.6	0.3
300	0.453	0.455	0.456	5.0	0.2

CORRECTION FOR SCATTERED ENERGY

To illustrate the accuracy of the correction method as applied to scattered energy, measurements were taken with a metal shutter on *n*-pentane at wave length 13.7 microns where sufficient intensity of scattered energy is present for tests. When the *n*-pentane band is tested by means of a transmitting LiF shutter, the measurements give a straight-line relationship. Thus the deviations obtained in the extinctions estimated from measurements made with a metal shutter may be readily attributed to scattered radiation within the instrument. As shown in Table II, the deviations between the corrected and theoretical extinctions are well within the experimental error of such measurements. The value of α as applied in this instance is 0.12.

Table III. Comparison of Observed, Corrected, and Theoretical Values of Extinction for Isobutane

(Wave length 8.4 microns, various pressures. Corrections for deviations attributed to band structure)

Partial Pressure, Mm. Hg	Extinction			Deviation from Theoretical, %	
	Observed	Corrected	Theoretical	Observed	Corrected
50	0.150	0.154	0.154	2.6	0.0
100	0.291	0.307	0.309	5.8	0.6
150	0.427	0.462	0.463	7.8	0.2
200	0.558	0.618	0.618	9.7	0.0
250	0.681	0.769	0.772	11.8	0.4
300	0.794	0.914	0.926	14.2	1.3

CORRECTION FOR BAND STRUCTURE

The deviations shown by isobutane at the wave length 8.4 microns are taken to show the application of the correction method to an instance where band structure is the factor involved. Isobutane does not exhibit pressure broadening of the band at this wave length (*5*) and the deviations are attributed to band shape (*3*). Both the scattered energy of the instrument on which these tests were made and the possibility of pressure broadening were checked in this laboratory for the wave-length setting used and found to be negligible. A value of 0.19 for the coefficient α is applied in this case. Results are shown in Table III.

It is clear that, within the experimental error, the proposed method applies to the correction of effects arising from band structure. This correction is characteristic of the band and therefore subject to experimental restrictions making modification of the calculation procedure necessary in some cases.

CORRECTION FOR MOLECULAR INTERACTION

The complex optical effects observed in the case of certain gases, of which methane is an example, have been repeatedly mentioned in the literature (*5*). It appears from these descriptions that certain absorption bands which fall into this category are not particularly desirable for analytical purposes where exact quantitative information is sought. However, the correction of this type of band is possible.

In cases where band broadening occurs, the absorption for the gas under investigation is influenced not only by the total pressure on the sample, but also by the nature of the molecules present, as is clearly shown in Figure 3. However, in such cases a single calibration curve for the absorber can be made to suffice by using a reference substance such as air as a diluting agent, so that the variations in the deviations due to the foreign substances present are made conveniently small.

Thus, if a sample is appropriately diluted and a fixed total pressure is used, Equation 2 holds for the correction listed under item 3. This may be visualized from the laboratory data shown in Table IV; α in this case is 7.59. The deviations of the corrected values are well within the accuracy of the measurements.

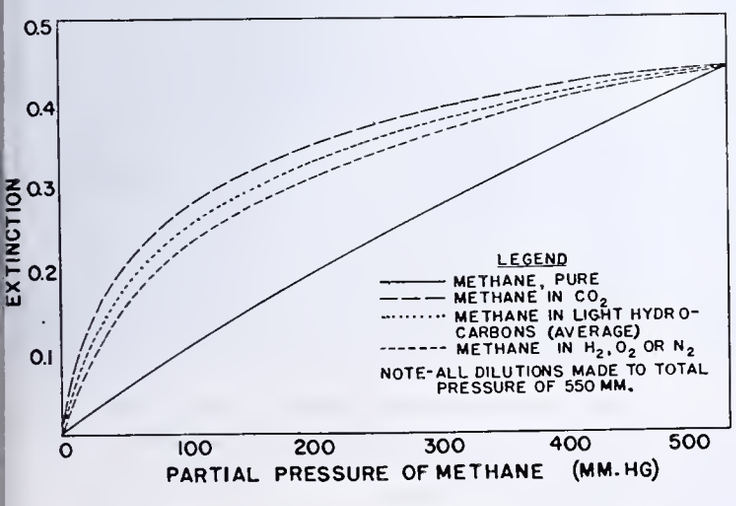

Figure 3. Extinction of Methane in Various Gases

Table IV. Comparison of Observed, Corrected, and Theoretical Values of Extinctions for Methane in Air

(Wave length 7.6 microns, total pressure of 400 mm. Corrections for deviations attributed principally to pressure broadening)

Partial Pressure, Mm. Hg	Extinction			Deviation from Theoretical, %	
	Observed	Corrected	Theoretical	Observed	Corrected
10	0.048	0.063	0.069	30.4	8.4
20	0.084	0.137	0.138	39.1	0.1
30	0.114	0.213	0.206	44.6	1.0
40	0.138	0.282	0.276	50.0	2.2
50	0.160	0.354	0.344	53.5	2.9
60	0.178	0.421	0.413	59.6	1.9
70	0.196	0.484	0.481	59.2	0.6
80	0.212	0.553	0.551	61.4	0.4
90	0.225	0.612	0.620	63.7	1.3

Table V. Comparison of Actual and Determined Compositions of Several Synthetic Blends

	Present, Mole %	Found, Mole %	Deviation, Mole %
	Sample 1		
Air	5.4	5.6	0.2
Methane	85.3	86.3	1.0
Ethylene	0.6	0.0	0.6
Ethane	4.8	5.1	0.3
Propylene	0.0	0.0	0.0
Propane	3.9	3.0	0.9
	Sample 2		
Methane	5.1	5.1	0.0
Ethylene	19.9	18.9	1.0
Ethane	28.4	29.5	1.1
Propylene	21.6	22.7	1.1
Propane	25.0	23.8	1.2
	Sample 3		
Air	11.7	11.7	0.0
Methane	38.2	39.7	1.5
Ethylene	9.9	8.7	1.2
Ethane	17.3	17.5	0.2
Propylene	8.1	7.9	0.2
Propane	14.8	14.5	0.3

ANALYSIS OF SYNTHETIC BLENDS

Table V shows the order of accuracy secured under actual analytical conditions. Synthetic blends were analyzed by diluting with air as described above. The hydrocarbon components were determined directly, while the air content of the original sample was obtained by difference. The deviations between the synthetic and calculated analyses are within the experimental error of the measurements.

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Identification of Organic Compounds by Use of Chromium Target X-Ray Diffraction Powder Patterns

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A study was made of the x-ray diffraction powder diagrams of a series of solid derivatives (anilides) of saturated aliphatic acids. It has been found that the use of chromium target x-radiation gives patterns of increased dispersion with the result that each member of the homologous series C_1 to C_{18} can readily be distinguished and identified. Structural isomers within the series may also be identified. Technical details on the use of chromium radiation for powder diffraction work are described. Tables of powder diffraction measurements are given.

THE use of x-ray diffraction patterns for the identification of crystalline material was outlined by Hull (*10*) in 1919 when he showed that a crystalline substance gives a diffraction pattern which is always the same for the same substance, and is sufficiently different from many of its chemical homologs to enable identification to be made. The application of the method has been mainly confined to inorganic compounds and minerals; the number of crystalline organic compounds recorded is relatively small. Of the one thousand patterns listed by Hanawalt, Rinn, and Frevel (*9*), forty-nine are of organic compounds. This is due in part to the complex nature of the powder patterns resulting from crystals of comparatively low symmetry. This condition should be improved if greater dispersion of the pattern were accomplished by the use of radiation of longer wave length and/or larger camera diameter and necessary refinement of collimator design. These difficulties were discussed at a recent meeting in England on the subject of physical methods of identification of materials, reported by Bannister (*2*). At this meeting Bunn suggested the use of larger camera diameter to give greater pattern dispersion.

The use of x-ray powder diffraction patterns for the identification of organic compounds poses certain unique problems. For example, the success or failure of the method in identifying individual members of a homologous series will depend upon the extent to which an additional methylene group affects the powder pattern of a typical member of the series. The differences between the patterns for each adjacent member must be sufficiently clear to establish identification. Similarly, the patterns for structural isomers must be distinctive. Rodgers (*2*) reported that in almost all cases powder patterns have proved exact enough to discriminate unambiguously between different organic compounds, even those of essentially similar chemical structure.

Recent work by McKinley, Nickels, and Sidhu on the identification of phenols (*13*) and by Clarke, Kaye, and Parks (*5*) on the identification of aldehydes and ketones by means of x-ray diffraction powder patterns clearly showed the applicability of the method. No published powder data, however, were available with which to evaluate the ability of the method to distinguish between each member of a homologous series, or of a number of structural isomers.

For the present work, the anilides of the saturated aliphatic acids were chosen. The identity of the anilides could readily be checked, as ample melting point data were available (*11*). These same anilides have been investigated by Robertson (*16*) for the purpose of relating melting point and molecular structure.

X-RAY APPARATUS AND TECHNIQUE

General Electric XRD Type I diffraction equipment was used in recording the powder patterns. Increased pattern dispersion was accomplished by the use of chromium K-α (λ = 2.285 kX) radiation. The effect on pattern dispersion of this longer wavelength radiation is illustrated in Figure 1. A very marked improvement in pattern dispersion and definition will be seen in the change from molybdenum K-α (λ = 0.708 kX) and copper K-α (λ = 1.537 kX). The same pattern dispersion could be accomplished by the use of larger camera diameters but the refinement in the collimator system required to give suitable definition of the pattern, together with the increased target to specimen and specimen to film distances, would require longer exposure times. The source of chromium radiation was a General Electric CA-6 Coolidge type tube with beryllium metal windows. The use of beryllium metal as the window of the tube is reported by Atlee (*1*) to give greatly increased useful intensity for wave length of x-radiation larger than 1.5 kX.

The filter used to pass essentially the K-α wave length was made from vanadium pentoxide (C.P., supplied by Eimer and Amend, New York, N. Y.) ground in cellulose acetate or nitrate cement (*14*). (Duco Household cement was found very satisfactory.) The mixture was thinned with amyl acetate and spread on glass to give a uniform film. After evaporation of the solvent, the film was stripped from the glass plate under water. The concentration found to give satisfactory removal of the Cr K-β lines was 15 mg. of vanadium pentoxide per square centimeter. This figure was determined by a series of powder photographs of sodium chloride taken with a range of concentrations of vanadium pentoxide in the filter. The concentration at which the (200) reflection due to K-β was barely detectable was considered satisfactory. The powder camera used was that of the General Electric XRD unit, the diameter being 143.2 mm. The specimen was mounted in a wedge, which was adjusted in the camera by sighting through the pinhole collimator to intercept one half of the beam. The tube was operated at a potential of 35 kv. and a current of 15 ma. Using Eastman Type K radiographic film the exposure time required was about 4 hours.

The relative intensities of the lines of the powder diffraction pattern should refer to randomly oriented powders. In the case of organic compounds which crystallize in needles or plates, this condition is experimentally difficult to obtain particularly in the case of wedge specimen mounts. The samples used were carefully ground in an agate mortar. In the case of one particularly plate-like material, propionanilide, a second sample was ground with Pyrex. No significant variation in relative line intensity was noted when compared to the previous photograph. The Pyrex adds considerably to the absorption in the specimen.

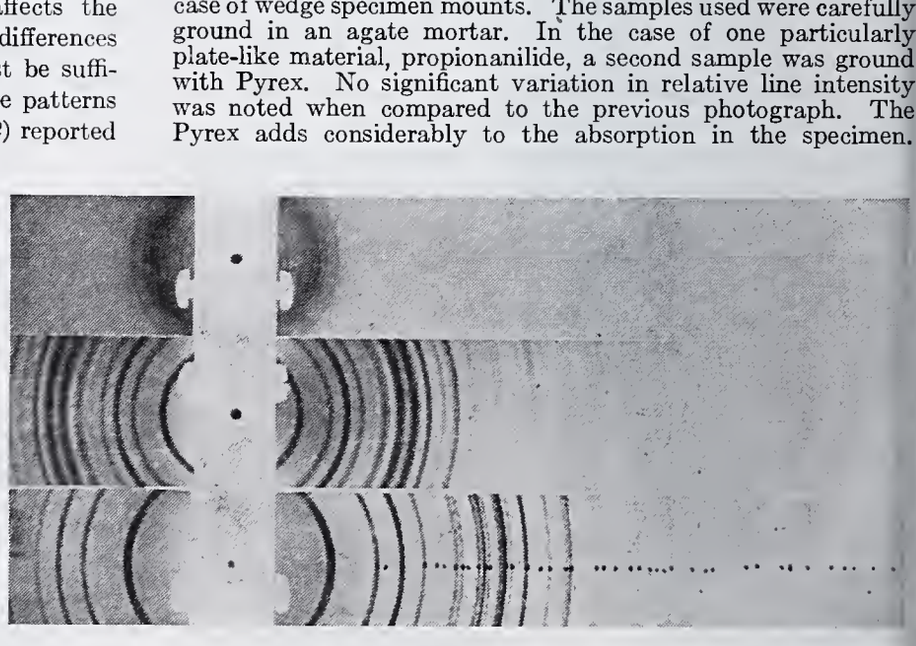

Figure 1. X-Ray Powder Diffraction Patterns of Acetanilide

Effect of change of x-ray wave length on pattern dispersion and resolution
Top. Mo K-α radiation, λ = 0.71 kX
Middle. Cu K-α, λ = 1.54 kX
Bottom. Chromium radiation, λ = 2.29 kX
Camera diameter 143.2 mm.

Table I. Melting Points of Anilides of Aliphatic Acids

Acid	No. of Carbon Atoms in Acid	Melting Point, ° C. (This Research)	Melting Point, ° C. (*11*)
ormic	1	47.6	50
cetic	2	113.6	114.1
ropionic	3	104	104–104.5, 105.6
-Butyric	4	93.5	92, 96, 97
sobutyric	4	102.5	104–105, 105
-Valeric	5	63	61–62, 62–63, 63
sovaleric	5	109	109.5, 109–110, 110
-Methylbutyric	5	109	105.5–106.5, 108, 110–111
ivalic	5	132	128, 132–133
-Caproic	6	94.5	92, 94–5, 96
socaproic	6	111	110.5, 111.5, 112
nanthic	7	64	64, 65, 69
aprylic	8	54	55, 57
elargonic	9	57.5	57
apric	10	68	70
ndecylic	11	70	71
auric	12	78	76.5, 78
lyristic	14	85	80–82, 84
almitic	16	91	90.5, 90.6
tearic	18	95	94, 95.5

indemann glass or beryllium metal powder might give improved esults.

PREPARATION OF ANILIDES

Formanilide was prepared by boiling an equimolar mixture of 7% formic acid and aniline until the temperature of the mixure reached 280° C. (*18*). The product was recrystallized from w-boiling petroleum ether. By refluxing together equimolar uantities of the acid and freshly distilled aniline for 2 to 3 hours ne following anilides were prepared: acetanilide (*19*), propinanilide (*6*), *n*-butyranilide, isobutyranilide, α-methylbutyrnilide, pivalanilide, and caproanilide (*12*). The anilides of -valeric, isovaleric, and isocaproic acids were prepared (*7*) by lding aniline to a benzene solution of the corresponding acid nloride in the proportion of 2 moles of aniline to 1 of acid chlode. In each case the mixture was brought to gentle reflux for minutes, then cooled, and the aniline hydrochloride removed by ltration. Evaporation of the filtrate gave the crude crystalline nilide. In the same way the anilides of undecylic, lauric (*4*), yristic, palmitic (*7*), and stearic acids were prepared (*8*). The nilides of enanthic, caprylic, pelargonic, and capric acids were btained by heating equimolar proportions of the acid and aniline sealed tubes at a temperature of 160° to 190° C. for 2 hours (*5*). In general it was found that the anilides of lower members the series (C_2 to C_5) could be crystallized readily from ethanolater mixtures, the intermediate members (C_6 to C_{11}) could be ystallized from petroleum ether, and the higher members (C_{12} to C_{18}) could be crystallized from benzene.

The melting points of the prepared anilides, as well as the alues given in the literature, are given in Table I.

DISCUSSION OF POWDER DIFFRACTION DATA

The powder diffraction data for the anilides of the normal liphatic acids from C_1 to C_{12}, inclusive, and of the even-numbered rbon acids C_{14} to C_{18} as well as for the iso acids C_4, C_5, and C_6 nd of two other structural isomers of the C_5 aliphatic acid are sted in Table II (p. 664). The intensities recorded were estiated from a visual examination of the film. The most intense nes of the patterns are listed in Table III and in Table IV the mpounds are listed in order of the strongest line of the patterns.

Examination of the powder diffraction data for unique characteristics by which identity of the compounds can be established shows that the patterns of the anilides of the normal acids $_1$ to C_8 are readily distinguished. The patterns of the even umbers of the series C_8 to C_{18} show a marked similarity, suggesg a uniformity of crystal structure which may be described as isostructural". Each pattern, however, has unique characteristics by which it can be distinguished; this is particularly true the longest *d* spacing recorded, which shows a measurable stepise change with increasing chain length of the acid. Only two ids with an odd number of carbon atoms greater than C_8 were amined. These indicated that a separate isostructural series is formed by these compounds. These results are in agreement with the findings of Slagle and Ott (*17*) in their x-ray investigation of the fatty acids.

A graphic representation of the melting point *vs.* carbon chain length of the compounds as given by Robertson (*17*) (see Figure 2) shows no regular relationship up to C_8. From C_8 to C_{18} the data show regularly increasing melting points with increasing carbon chain length. This result is in conformity with the isostructural relationship found in the examination of the powder data.

The effect of structural isomerism on the powder diffraction pattern was examined in the case of the normal and isoisomers of butyric, valeric, and caproic acids. The pattern of the straight-chain derivative could be readily distinguished from that of its structural isomer. In the case of the C_5 acid four structural isomers were examined: *n*-valeric, isovaleric, α-methylbutyric, and pivalic acids. The diffraction patterns of the anilides of these acids could be readily distinguished.

Since the x-ray diffraction powder pattern is specific for each polymorphic form of a substance, the possible existence of such forms should be considered. From the x-ray diffraction examination no evidence was observed of polymorphic changes in these compounds. All samples were crystallized from solvents, at room temperature. These conditions would tend to give a product stable at room temperature. Patterns taken at intervals of several months on the same specimen showed no change. The

Table III. Four Strongest Lines of Powder Diffraction Patterns of Anilides of Some Saturated Aliphatic Acids

Name	No. of Carbon Atoms in Aliphatic Acid	1st Line	2nd Line	3rd Line	4th Line
Formanilide	1	3.61	3.75	3.09	5.16
Acetanilide	2	9.44	5.92	3.64	3.41
Propionanilide	3	3.75	6.17	8.59	4.46
n-Butyranilide	4	4.05	5.05	5.65	3.55
Isobutyranilide	4	7.09	4.05	5.72	4.47
n-Valeranilide	5	4.77	3.64	10.25	5.50
Isovaleranilide	5	7.41	4.13	9.95	5.00
d,l-α-Methylbutyranilide	5	7.28	4.17	3.28	5.17
Pivalanilide	5	7.14	4.20	4.62	5.00
n-Caproanilide	6	3.82	3.55	5.55	4.77
Isocaproanilide	6	4.21	7.51	4.80	3.53
Enanthanilide	7	4.70	4.15	4.00	3.78
Caprylanilide	8	16.4	4.03	3.94	8.06
Pelargonanilide	9	17.0	8.46	4.49	3.95
Capranilide	10	19.1	4.05	9.12	3.93
Undecylanilide	11	4.05	4.25	3.64	17.4
Lauranilide	12	3.86	20.7	3.75	3.61
Myristanilide	14	4.36	3.85	11.6	3.73
Palmitanilide	16	4.40	3.85	3.75	3.63
Stearanilide	18	4.41	3.85	3.74	3.65

Table IV. Data Arranged in Order of Strongest Line of Diffraction Pattern

1st Line	2nd Line	3rd Line	4th Line	No. of Carbon Atoms in Aliphatic Acid	Name
3.61	3.75	3.09	5.15	1	Formanilide
3.75	6.17	8.59	4.46	3	Propionanilide
3.92	3.55	5.55	4.77	6	*n*-Caproanilide
3.86	20.7	3.75	3.61	12	Lauranilide
4.05	5.05	5.65	3.55	4	*n*-Butyranilide
4.05	4.25	3.64	17.4	11	Undecylanilide
4.21	7.51	4.80	3.53	6	Isocaproanilide
4.36	3.85	11.6	3.73	14	Myristanilide
4.40	3.85	3.75	3.63	16	Palmitanilide
4.41	3.85	3.74	3.65	18	Stearanilide
4.70	4.15	4.00	3.78	7	Enanthanilide
4.77	3.64	10.25	5.50	5	*n*-Valeranilide
7.09	4.05	5.72	4.47	4	Isobutyranilide
7.14	4.20	4.62	5.00	5	Pivalanilide
7.28	4.17	3.28	5.17	5	*d,l*-α-Methylbutyranilide
7.41	4.13	9.95	5.00	5	Isovaleranilide
9.44	5.92	3.66	3.41	2	Acetanilide
16.4	4.03	3.94	8.06	8	Caprylanilide
17.0	8.46	4.49	3.95	9	Pelargonanilide
19.1	4.05	9.12	3.93	10	Capranilide

Table II. X-Ray Diffraction Data

Formanilide, C_1

d	I/I_1
14.0	0.50
7.28	0.15
7.03	0.20
6.19	0.05
5.94	0.10
5.61	0.25
5.32	0.40
5.16	0.70
4.86	0.40
4.69	0.70
4.33	0.70
4.13	0.10
3.81	0.50
3.75	0.80
3.61	1.00
3.52	0.02
3.45	0.05
3.25	0.40
3.09	0.80
2.99	0.10
2.93	0.05
2.81	0.15
2.65	0.05
2.55	0.05
2.52	0.05
2.38	0.05
2.34	0.05
2.30	0.05
2.24	0.05
2.18	0.05
2.04	0.05
2.01	0.05

Acetanilide, C_2

d	I/I_1
9.44	1.00
6.66	0.50
6.33	0.05
5.92	0.80
5.09	0.50
4.83	0.05
4.67	0.10
4.40	0.30
4.28	0.30
4.23	0.02
4.03	0.20
3.95	0.30
3.86	0.20
3.66	0.60
3.58	0.30
3.41	0.60
3.26	0.15
3.17	0.20
3.02	0.05
2.99	0.25
2.79	0.15
2.74	0.05
2.65	0.10
2.56	0.15
2.51	0.15
2.46	0.10
2.42	0.05
2.38	0.02
2.35	0.20
2.25	0.20
2.19	0.05
2.16	0.10
2.04	0.10
2.01	0.10
1.93	0.10
1.90	0.10

Propionanilide, C_3

d	I/I_1
8.59	0.60
6.17	0.80
4.86	0.40
4.46	0.60
3.75	1.00
3.21	0.60
3.08	0.02
2.82	0.10
2.70	0.10
2.59	0.05
2.56	0.10
2.40	0.05
2.26	0.05
2.20	0.10
2.16	0.10
1.93	0.05

n-Butyranilide, C_4

d	I/I_1
10.25	0.05
8.64	0.05
7.73	0.05
6.94	0.05
5.65	0.30
5.05	0.50
4.78	0.10
4.67	0.10
4.45	0.07
4.05	1.00
3.92	0.15
3.73	0.15
3.47	0.10
3.35	0.30
3.09	0.20
3.00	0.07
2.64	0.02
2.47	0.05
2.04	0.10

Isobutyranilide, C_4

d	I/I_1
9.94	0.40
7.09	1.00
5.72	0.60
5.00	0.50
4.77	0.50
4.47	0.60
4.20	0.10
4.05	0.80
3.86	0.20
3.62	0.05
3.47	0.02
3.35	0.30
3.18	0.20
3.11	0.15
2.99	0.02
2.29	0.05
2.25	0.02
2.05	0.07

d,l-α-Methylbutyranilide, C_5

d	I/I_1
10.27	0.20
7.28	1.00
6.58	0.05
5.80	0.60
5.17	0.70
4.93	0.30
4.77	0.30
4.63	0.70
4.55	0.20
4.46	0.05
4.33	0.05
4.17	1.00
3.95	0.30
3.70	0.30
3.43	0.60
3.28	0.80
3.12	0.40
3.01	0.15
2.95	0.10
2.89	0.15
2.71	0.10
2.61	0.10
2.46	0.10
2.33	0.15
2.22	0.05
2.20	0.05
2.09	0.10

Isovaleranilide, C_5

d	I/I_1
9.95	0.80
7.41	1.00
7.00	0.02
5.85	0.50
5.00	0.70
4.75	0.70
4.59	0.05
4.13	1.00
3.88	0.20
3.70	0.20
3.47	0.20
3.34	0.70
3.19	0.70
3.07	0.20
2.66	0.30
2.45	0.05
2.29	0.05
2.11	0.05
2.04	0.10

n-Valeranilide, C_5

d	I/I_1
10.25	0.60
6.48	0.50
5.50	0.60
5.25	0.60
4.77	1.00
4.59	0.05
4.44	0.05
4.25	0.10
3.78	0.15
3.64	1.00
3.50	0.20
3.39	0.30
3.33	0.10
3.00	0.05
2.88	0.05
2.38	0.10
2.04	0.10

Pivalanilide, C_5

d	I/I_1
7.14	1.00
6.10	0.01
5.83	0.20
5.21	0.20
5.00	0.50
4.62	0.60
4.51	0.30
4.41	0.15
4.20	1.00
4.04	0.10
3.37	0.20
3.29	0.20
3.13	0.10
2.98	0.02
2.91	0.05
2.85	0.02
2.74	0.02
2.65	0.02
2.44	0.02
2.39	0.02
2.33	0.05
2.26	0.02
2.23	0.05

n-Caproanilide, C_6

d	I/I_1
10.8	0.15
7.57	0.05
6.85	0.20
5.92	0.05
5.55	0.60
4.77	0.60
4.67	0.10
4.31	0.10
3.82	1.00

Isocaproanilide, C_6

d	I/I_1
14.8	0.20
7.51	0.80
5.92	0.30
5.59	0.05
5.16	0.30
4.80	0.80
4.36	0.30
4.21	1.00
4.05	0.10

Enanthanilide, C_7

d	I/I_1
8.13	0.20
7.21	0.30
5.88	0.60
5.72	0.10
5.59	0.10
5.16	0.02
4.70	1.00
4.47	0.02
4.15	0.80

n-Caproanilide, C_6 (*Contd.*)

d	I/I_1
3.55	0.80
3.46	0.10
3.22	0.10
2.98	0.10
2.89	0.10
2.51	0.10
2.45	0.10
2.17	0.10
2.04	0.10

Isocaproanilide, C_6 (*Contd.*)

d	I/I_1
3.80	0.10
3.53	0.50
3.45	0.10
3.31	0.50
3.12	0.15
2.93	0.02
2.89	0.02
2.83	0.05
2.78	0.05
2.72	0.02
2.56	0.05
2.53	0.05
2.45	0.10
2.36	0.10
2.29	0.05
2.25	0.05
2.19	0.10
2.13	0.15
1.99	0.10
1.94	0.05
1.91	0.05

Enanthanilide, C_7 (*Contd.*)

d	I/I_1
4.00	0.80
3.78	0.80
3.59	0.60
2.27	0.10
2.21	0.10

n-Caprylanilide, C_8

d	I/I_1
16.4	1.00
12.9	0.20
11.1	0.20
10.2	0.20
8.06	0.50
6.65	0.10
5.59	0.20
4.77	0.10
4.38	0.20
4.22	0.20
4.03	0.60
3.94	0.60
3.82	0.20
3.66	0.20
3.22	0.05
3.14	0.15
3.06	0.10
2.89	0.05
2.57	0.05
2.39	0.05
2.10	0.05

Pelargonanilide, C_9

d	I/I_1
17.0	1.00
11.5	0.10
10.6	0.05
8.46	0.80
5.92	0.20
4.49	0.50
4.40	0.20
4.25	0.10
4.05	0.40
3.95	0.50
3.78	0.05
3.68	0.05
3.14	0.10
2.05	0.15

Capranilide, C_{10}

d	I/I_1
19.1	1.00
16.1	0.02
12.4	0.10
10.8	0.05
9.12	0.80
6.40	0.15
4.86	0.30
4.45	0.50
4.23	0.50
4.05	1.00
3.93	0.60
3.81	0.40
3.67	0.30
3.50	0.10
3.14	0.30
2.98	0.10

Undecylanilide, C_{11}

d	I/I_1
17.4	0.20
8.89	0.20
5.98	0.15
4.66	0.02
4.44	0.05
4.36	0.05
4.25	0.50
4.05	1.00
3.89	0.20
3.64	0.30
3.42	0.15
3.29	0.02
3.21	0.10
3.14	0.05
3.06	0.05
3.02	0.05
2.81	0.05
2.45	0.10
2.35	0.02
2.30	0.02
2.29	0.02
2.24	0.05
2.18	0.05
2.07	0.02
2.04	0.05
2.00	0.05

Lauranilide, C_{12}

d	I/I_1
20.7	0.60
10.30	0.15
9.27	0.30
8.13	0.30
6.94	0.02
5.37	0.15
5.17	0.10
4.52	0.10
4.38	0.40
4.05	0.30
3.86	1.00
3.75	0.50
3.61	0.50
3.14	0.20
3.07	0.02
2.33	0.05
2.30	0.05

Myristanilide, C_{14}

d	I/I_1
23.4	0.30
12.8	0.05
11.6	0.50
9.94	0.30
8.78	0.05
7.65	0.30
7.14	0.10
6.01	0.20
4.86	0.30
4.61	0.05
4.36	1.00
4.03	0.40
3.85	1.00
3.73	0.50
3.62	0.50
3.37	0.02
3.14	0.20
2.43	0.10
2.31	0.05
2.18	0.02

Palmitanilide, C_{16}

d	I/I_1
12.6	0.50
10.3	0.10
9.12	0.02
8.21	0.30
7.34	0.05
6.55	0.15
5.31	0.15
4.86	0.20
4.56	0.05
4.40	1.00
4.23	0.10
4.13	0.05
4.00	0.15
3.85	1.00
3.75	0.60
3.63	0.60
3.14	0.10
3.06	0.02
2.42	0.10
2.35	0.02
2.30	0.05
2.11	0.02
2.07	0.02
2.04	0.02

Stearanilide, C_{18}

d	I/I_1
16.4	0.20
14.0	0.20
12.2	0.02
10.9	0.02
9.49	0.05
8.78	0.05
7.79	0.02
7.09	0.02
6.75	0.01
5.74	0.01
5.25	0.01
4.81	0.05
4.41	1.00
4.01	0.20
3.85	1.00
3.74	0.50
3.65	0.50
3.14	0.10
2.43	0.10
2.08	0.02
2.05	0.10

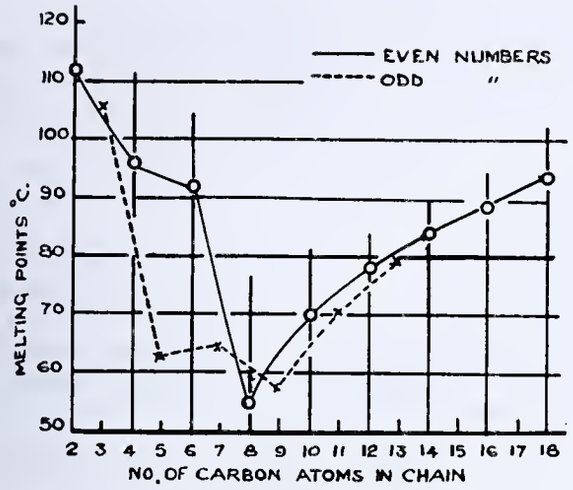

Figure 2. Effect of Chain Length on Melting Point of Anilides of Aliphatic Acids

Data from Robertson (*16*)

optical studies of Bryant (*3*) on the *p*-bromoanilides of the aliphatic acids showed metastable polymorphic forms which, however, were evident only in crystallizations from melt.

It was noted that extreme purity of the crystalline samples was not necessary in obtaining distinctive diffraction patterns. For instance, a crude sample of acetanilide, melting 5° below that of National Bureau of Standards microanalytical acetanilide, gave a pattern identical with that of the purer sample. This is an indication that great care in recrystallizing a derivative until a constant melting point is reached may not always be necessary for identifications when the x-ray diffraction method is employed. This observation is in agreement with the authors' experience in the wider application of the method.

SUMMARY

Using chromium target x-radiation, the powder diffraction patterns for the anilides of the normal saturated fatty acids (C_1 to C_{18}) were shown to be sufficiently characteristic to enable individual identification. The anilides of structural isomers of several of the fatty acids gave distinctive diffraction patterns. Extreme purity of the crystalline derivatives was found to be unnecessary in obtaining characteristic patterns.

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Determination of Hydrogen Sulfide in Gases

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Two colorimetric methods have been developed for the analysis of traces of hydrogen sulfide in gases. The hydrogen sulfide is first absorbed in a caustic solution, and in the more sensitive method is converted to bismuth sulfide. The concentration of the resulting suspension is determined by means of a spectrophotometric measurement. In the absence of a spectrophotometer the sulfide is made to react with a uranyl-cadmium reagent and analyzed by visual comparison in a chromometer. With the spectrophotometric method as little as 7 micrograms of hydrogen sulfide may be determined with a precision of ±10%. For larger samples the precision improves to ±3%. The chromometer technique requires five times as much sulfide for equal precision, but still is far more sensitive than titration.

THE sensitivity of even the elaborate titration technique described by Shaw (*3*) for the determination of hydrogen sulfide is inadequate for such special applications as the study of catalyst poisons, particularly where the lack of sensitivity cannot conveniently be overcome by increasing the size of the sample to be analyzed. In a more effective procedure for the determination of traces of hydrogen sulfide, presented by Moses and Jilk (*2*), a photoelectric cell measures the degree of darkening of a lead acetate–impregnated tape through which the gases are passed. The present paper describes another approach to the problem whereby as little as 1.4 micrograms of hydrogen sulfide in aqueous solution can be detected optically. Two procedures were developed specifically to meet the problems of the identification and determination of organic sulfur compounds present as impurities in commercial gases and solvents. Their application to the solution of these problems will be described in succeeding papers.

Both of the analytical methods described employ a solution of the hydrogen sulfide in 6% aqueous sodium hydroxide. For the applications under consideration this involves scrubbing the sulfide-bearing gas with caustic. In the more sensitive of the two analytical methods the sulfide in solution is converted to bismuth sulfide and the concentration of the resulting suspension is determined by measuring the transmission of monochromatic light with a spectrophotometer. The precision of the analysis is determined by the amount of hydrogen sulfide collected in the absorbing solution. The precision is ±10% when 7 micrograms of hydrogen sulfide are collected. This corresponds to the amount of sulfur in 0.1 cubic foot of gas containing sulfur in a concentration of 0.11 grain per 100 cubic feet (1 grain per 100 cubic feet = 22.9 micrograms per liter). With larger concentrations of sulfur or a larger sample, a precision of ±3% can be achieved.

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The second method is useful when a spectrophotometer is not available. In this case cadmium sulfide is precipitated in the presence of uranyl ion and the sulfide concentration is then determined by matching of color and opacity against a blank in a visual comparometer. The sensitivity is approximately one fifth that of the spectrophotometric method, but is still far superior to that experienced in titration methods.

GENERAL PROCEDURE

Reagents. The following solutions are required.

Caustic Solution, 6% by weight. Dissolve 1130 grams of reagent grade sodium hydroxide in 10 liters of distilled oxygen-free water and dilute to 18 liters.

Bismuth Reagent. Dissolve 42.8 grams of c.p. bismuth nitrate pentahydrate in 3 liters of glacial acetic acid and dilute with 15 liters of distilled water.

Uranyl-Cadmium Reagent. Dissolve 44.4 grams of uranyl nitrate hexahydrate and 31.4 grams of cadmium acetate dihydrate in 20 liters of distilled water plus 4 liters of glacial acetic acid (all c.p.).

Standard Sulfide Solutions for Calibration Purposes. Prepare fresh each day by dissolving approximately 3 grams of c.p. sodium sulfide nonahydrate in 1 liter of the 6% caustic solution. Determine concentration by iodometric titration (*3*). Dilute aliquots with 6% caustic to provide solutions of known concentrations for the calibrations.

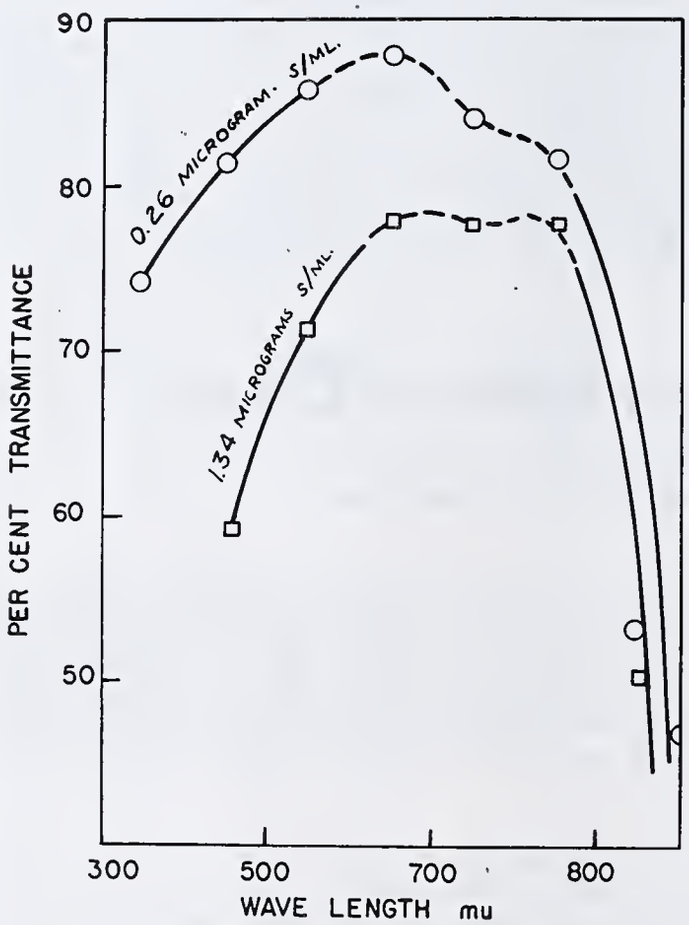

Figure 1. Light Absorption by Bismuth Sulfide Suspension

Scrubbing Hydrogen Sulfide from a Gas Stream. Both procedures employ a caustic solution of sulfide ion obtained by scrubbing the gas to be analyzed. To avoid oxidation of the sulfide ion the gas must be free of oxygen, but hydrogen, nitrogen, carbon monoxide, methane, ethylene, and up to 2% carbon dioxide do not interfere. Up to 1% oxygen in the gas to be analyzed is automatically removed by the procedure for converting organic sulfur compounds to hydrogen sulfide (*1*). Hydrogen sulfide is readily and completely absorbed from a gas by bubbling through a 6% caustic solution. For the chromometer method a Milligan gas-washing bottle containing 100 ml. of 6% caustic has been found very satisfactory. The spectrophotometric analysis requires only 10 ml. of the caustic solution. In order to take full advantage of the smaller reagent volume requirement in terms of minimum gas sample, bubble bottles were employed with capacities as low as 10 ml. With either the Milligan or the bubble bottles, gas rates of 0.6 liter per minute were used without sulfide leakage. After a sufficient quantity of gas is scrubbed the solution is mixed and an aliquot taken for analysis.

General Precautions. The methods are extremely sensitive and consequently are very susceptible to serious errors from accidental contamination. No grease of any type should be used on stopcocks and ground-glass joints, since it causes a discoloration of the caustic. Glassware should be thoroughly cleaned, preferably with acetone or similar organic solvent followed by several water rinses.

Since sulfides are readily oxidized by dissolved oxygen, all solutions are deoxidized and transferred where possible under a blanket of nitrogen. The short time of exposure in the final mixing of reagents has a negligible effect.

The analysis requires the addition of either the bismuth or the uranyl reagent to the caustic solution of the sulfide. The order of mixing and the time of standing between preparation and analysis must be rigidly standardized. When the reagents are mixed, heat of neutralization is evolved, effervescence may occur, and crystal growth or agglomeration begins. These changes are rapid at first but become slow after 3 to 5 minutes. Despite the heat of reaction it was not found necessary to thermostat the solution. In the course of several hundred determinations by each method, no tendency for the precipitate to settle could be observed during the 10 minutes necessary for the analysis. (A private communication from J. K. Fogo, who was kind enough to check the spectrophotometric procedure, states that difficulty was encountered from a tendency of the bismuth sulfide to precipitate before the light transmittance could be measured. His suggestion that all trouble from this source may be avoided by adding 0.2% clear Knox gelatin to the bismuth reagent appears to have real value.)

The exact concentration of reagents is not critical; however, they must be so balanced that the final solution is weakly acid.

SPECTROPHOTOMETRIC METHOD

Equipment. A Coleman Model 10S double monochromator spectrophotometer was employed, in conjunction with a Coleman 310 electrometer. The cuvettes were cylindrical Pyrex tubes 16 mm. in inside diameter.

Procedure. To the 6% sodium hydroxide solution containing the sulfide, an equal volume of the bismuth nitrate–acetic acid reagent is added. The product is weakly acid, so that carbon dioxide is liberated from any carbonate present. The solution is mixed by bubbling deoxidized nitrogen (purchased as such in standard cylinders from Air Reduction Sales Co.) through it for 30 seconds and then is allowed to stand for exactly 5 minutes. A portion of this sample (10 ml.) is poured into the spectrophotometer cuvette. The spectrophotometer is balanced at an arbitrary 100% light transmission in the absence of the cuvette, and 7 minutes from the time of precipitation the per cent transmittance of sample is determined with the cuvette in place. The sulfide concentration is then read directly from a calibration chart.

When sampling a gas containing a very low concentration of hydrogen sulfide it is necessary to pass enough gas through the

Table I. Sample Calibration Data for Spectrophotometric Method

Micrograms/Ml.[a]	% Transmittance
0	84.1
0.292	69.6
0.292	68.8
0.292	68.0
0.73	52.7
0.73	51.1
0.73	51.1
1.47	31.5
1.47	32.4

[a] Diluted from standard solution containing 1.47 grams of sulfur per ml. as determined iodometrically.

Table II. Analysis of Hydrogen Sulfide-Bearing Gas by the Spectrophotometric and Colorimetric Methods

Run No.	H_2S Concentration (Arbitrary Units) Spectrophotometer	Colorimeter
1	17.1	17.7
2	18.0	17.7
3	20.6	22.9
4	20.6	21.2
5	22.4	20.5
6	20.6	20.5
7	20.6	20.5
7A	2.5	2.4
8	22.2	21.2
9	18.6	18.3
10	12.0	11.0
11	13.2	12.0

minimum quantity of caustic (10 ml.) to give between 6 and 40 micrograms of sulfur. When more concentrated gases are being analyzed, larger volumes of caustic solution can be employed or the sample can be diluted with fresh caustic, so that the final 10-ml. sample contains the amount of sulfur specified above.

Calibration. The calibration chart is made initially by repeating the above determinations on solutions of 6% sodium hydroxide containing known sulfide concentrations prepared from the standard solution. When the sulfide concentration of these solutions is plotted against the logarithm of the per cent transmittance the relationship is linear. A typical set of calibration data is shown in Table I. The slope of the line remains the same from day to day but the intercept at zero sulfide concentration shifts slightly from 85% transmittance, presumably because of changes in the reagents and photometric system. Once the slope is established, therefore, it is sufficient to calibrate the unit by mixing equal volumes of the sulfide-free caustic and bismuth reagents and measuring per cent transmittance to determine the zero point.

Selection of Reagent and Light Wave Length. Cadmium, lead, and bismuth were examined as reagents for the sulfide precipitation. Of these, bismuth sulfide produced the greatest changes in per cent transmittance of light for a given change in sulfide concentration. Consequently bismuth was selected for further study. The variation in transmittance of light as a function of wave length for aqueous suspensions of bismuth sulfide of two different concentrations is shown in Figure 1. It is seen that the greatest sensitivity is obtained at the shortest wave length which could be reached with the spectrophotometer employed, 350 millimicrons. All analyses were therefore performed with light of this wave length (actually 350 to 355 mμ). It is possible that even shorter wave lengths would have given greater sensitivity.

VISUAL METHOD

Equipment. During a period when the spectrophotometer was not available, visual comparisons were made by means of a Saybolt Standard universal chromometer No. 2895. A standard chromometer comparator tube was used for the blank, while the second tube was of special design with a leveling tube and plunger (Figure 2, an adaptation of the principle employed in the Kennicott, Campbell and Hurley colorimeter, Eimer & Amend Catalog No. 7-155). The light source was a G.E. Type H-3 mercury vapor lamp mounted in a Bausch & Lomb Type B adjustable microscope lamp housing. Polished standard thickness Corning filters 30 and 512, 5 cm. (2 inches) square, were mounted between the light source and the chromometer. A scale 10 units high (40 cm.) is mounted behind the tubes to measure the liquid height.

Procedure. A blank solution is prepared by mixing equal volumes of the 6% caustic and the uranyl-cadmium reagent. A sufficient quantity of this solution is put into the blank tube to fill it just to the "10" mark on the scale (approximately 120 ml.). An aliquot of the standard sulfide solution containing 30 to 200 micrograms of sulfur is diluted with 6% sodium hydroxide to 70 ml. and mixed by means of a stream of nitrogen. After mixing, 70 ml. of the uranyl reagent are added and again the solution is agitated with nitrogen. The solution is then poured into the special comparator tube and allowed to stand 3 minutes before balancing. The analysis is completed by adjusting the balancing plunger in the side arm of the comparator tube until the color and intensity of the sulfide solution match those in the blank as nearly as possible. The procedure is repeated with sulfide aliquots of different sizes.

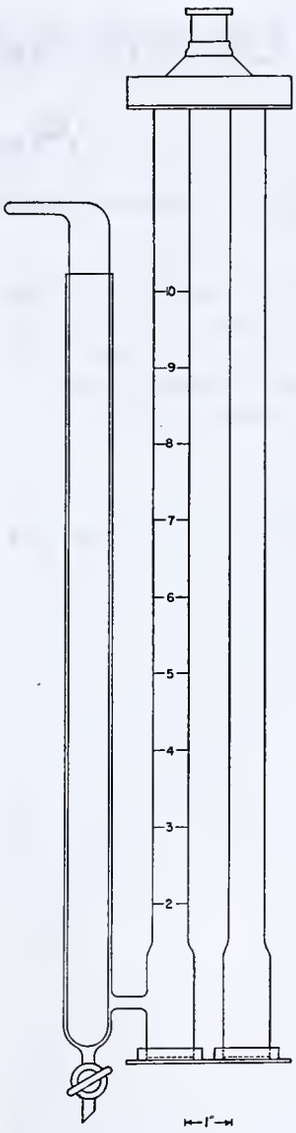

Figure 2. Chromometer Comparison Tube

If the logarithm of the balancing height is plotted against the sulfide content, a straight line is obtained except for a gentle curve near the origin. A new curve must be prepared each day because of uncontrollable variations. Unknown sulfide solutions are analyzed by the same technique. When collecting a sample sufficient gas must be passed through the minimum volume of caustic (70 ml.) to give at least 30 micrograms of sulfur in order to fall in the range of the chromometer. If more than 200 micrograms of sulfur is collected, an aliquot is taken.

Although the balance point is readily reproducible, a personal factor is definitely present. For maximum precision each operator should prepare his own calibration curves. Eye fatigue is a problem if a large number of analyses are involved.

A comparison of analyses by the spectrophotometric and the colorimetric methods is shown in Table II where the off gas from an experiment was analyzed simultaneously by both methods.

Discussion of Visual Technique. The function of the uranyl ion in the solution is not clear, but it greatly improves the accuracy of visual comparison over that with cadmium sulfide alone. The advantage gained by the use of the uranyl-cadmium reagent is limited to measurements in the chromometer and does not appear in spectrophotometer studies. The two filters mounted on the mercury lamp transmit only the green and blue components of the light, both of which are partially transmitted by the sulfide-free solution. When sulfide is added, the green transmission is unaffected but some blue is absorbed. The degree of absorption is a function of the concentration and height of the column of sulfide solution. A nearly exact color balance can be obtained between a sulfide-free and a sulfide-containing solution by adjusting the height of the latter.

The specially designed comparator tube with a plunger to balance the heights of liquid is more convenient to use and permits higher precision than comparators which depend upon stopcocks for establishing liquid levels. Very little extra liquid is required and the plunger permits approaching balance from either side until the operator is certain that the best match has been achieved.

ACKNOWLEDGMENT

The authors are indebted to numerous members of the laboratory staff for assistance in carrying out the work and in the preparation of this paper.

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Conversion of Organic Sulfur to Hydrogen Sulfide for Analysis

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Organic sulfur compounds are quantitatively converted to hydrogen sulfide by passing with hydrogen over alumina at 900° C. The technique has made possible the analysis of traces of organic sulfur compounds in gases or liquids when used in conjunction with sufficiently sensitive methods for hydrogen sulfide analysis.

MODERN catalytic processes require raw materials possessing a high degree of purity. Even traces of hydrogen sulfide or organic sulfur compounds in gases or liquids are detrimental to many catalytic reactions. In order to control and eliminate these catalyst poisons, highly sensitive analytical devices are essential. Several satisfactory procedures for hydrogen sulfide determinations are available, such as the colorimetric methods described in a recent paper from this laboratory (*2*), or the lead acetate–impregnated tape method of Moses and Jilk (*5*). For specific organic sulfur compounds there is the highly sensitive isatin test for thiophene (*1*) and the ultraviolet absorption technique for carbon disulfide (*7*). However, the commonly used combustion technique for total organic sulfur, involving converting the sulfur to sulfate followed by gravimetric or turbidimetric analysis (*3*, *6*), is very difficult to apply in the region of extremely low sulfur content where the reagents may contain more sulfate than the sample being analyzed.

The purpose of the present study has been the development of a technique for complete conversion of all volatile organic sulfur compounds present to hydrogen sulfide, after which any sufficiently sensitive procedure for hydrogen sulfide can be applied. A modification of the method presented by Jilk (*4*) was selected after a survey of various alternative procedures. In Jilk's method the gas containing organic sulfur compounds is freed from oxygen, humidified, and passed over activated alumina at 500° C. By raising the catalyst temperature from 500° to 900° C. as much as 1% oxygen in the gas was found to have no effect on the results. Humidification of the gas was not necessary, and complete conversion of methyl mercaptan and thiophene to hydrogen sulfide was obtained, whereas at 500° C. only 60 and 20% conversions were obtained, respectively. At 900° C. carbonyl sulfide, carbon disulfide, methyl mercaptan, thiophene, methyl thiocyanate, and sulfur dioxide were tested and found to be completely converted to hydrogen sulfide when passed over activated alumina in the presence of excess hydrogen. No other sulfur compounds were tested.

PROCEDURE FOR GASES

The gases, containing organic sulfur and excess hydrogen, are passed over 6 ml. of 14- to 20-mesh alumina (desulfurized Alorco Grade A, or gel prepared by precipitation from aluminum nitrate solution with ammonia) in a 10-mm. inside diameter quartz or Vycor tube heated to 900° in a vertically mounted split electric furnace. All connecting lines are glass; rubber joints, where unavoidable, are boiled in caustic to remove sulfur, and a minimum of rubber surface is exposed since rubber reversibly absorbs sulfur compounds. Gas rates up to 0.6 liter per minute were found permissible. To avoid errors caused by absorption on the catalyst, it is desirable to equilibrate the catalyst with the gas for at least 30 minutes; with small samples the catalyst may be purged with pure hydrogen for one hour before the analysis, and the adsorbed sulfur removed by another 1-hour purge to conclude the analysis. The hydrogen sulfide–bearing gas from the converter passes through a Milligan bubbler containing 6% caustic solution. The hydrogen sulfide absorbed is analyzed colorimetrically as described in an earlier paper from this laboratory (*2*).

PROCEDURE FOR LIQUIDS

The liquid to be analyzed is placed in a suitable bubbler and sulfur-free hydrogen is passed through it at a rate of approximately 0.6 liter per minute. The volume of liquid is adjusted to give a suitable quantity of hydrogen sulfide for subsequent analysis. The temperature of the bubbler is chosen to give a convenient rate of evaporation, and hydrogen is passed through it until the sample is completely volatilized. The gas stream is passed over alumina at 900° C. and the hydrogen sulfide collected in caustic, just as in the procedure for gases. The hydrogen stream is continued for another hour to flush the system and recover all sulfur adsorbed by the catalyst. Alternatively, nonvolatile or high-boiling liquids can be stripped of their volatile sulfur content with hydrogen by prolonged bubbling without complete vaporization of the liquid. In this case the analysis would necessarily be for volatile sulfur only. The time required is best determined by experiment, since it depends on the volatility of the sulfur compounds present.

Some vapors—for instance, benzene—deposit carbon on the catalyst. If the deposit becomes heavy the activity of the catalyst is lowered. Should this tendency exist, a very small sample must be analyzed or the catalyst must be regenerated periodically during the determination. Carbon deposits also increase the adsorption of sulfur on the catalyst and make necessary prolonged purging with hydrogen at the end of the analysis. Complete regeneration is accomplished by passing air over the catalyst for 5 minutes at 900° C. The catalyst is purged of sulfur before each regeneration. The regenerating air is by-passed around the absorbing solution to avoid oxidation of sulfide.

DEMONSTRATION OF CONVERSION TO HYDROGEN SULFIDE

Activated alumina (Alorco Grade A) or precipitated aluminum oxide when charged as 14- to 20-mesh granules in a quartz tube was found to give complete conversion at 900° C. at a space velocity of 3000 hours^{-1}. The hydrogenation was demonstrated for carbonyl sulfide, carbon disulfide, methyl mercaptan, methyl thiocyanate, thiophene, and sulfur dioxide, as shown in Table I. The determinations were made by introducing a measured volume of the gas or a weighed amount of the liquid into the gas stream. The actual concentration was therefore indefinite. It was found impractical to make up homogeneous gas samples for this purpose

Table I. Conversion of Organic Sulfur to Hydrogen Sulfide over Alumina at 900° C.

Sulfur Compound	Space Velocity	Average Sulfur Concentration		Carrier Gas	Converted[a] to H_2S
	Hours^{-1}	*Micrograms/liter*	*Grains/100 cu. ft.*		%
COS	2800	1260	55.7	H_2	97
	2800	1260	55.7	33CO-67H_2	96
	2800	500	21.9	H_2	104
	17,000	96	4.2	33CO-67H_2	100
	Empty tube	440	19.4	33CO-67H_2	100
CS_2	3000	530	23.0	H_2	96
CH_3SH	3000	1010	44.5	33CO-67H_2	109
	3000	1260	55.7	H_2	100
CH_3SCN	3000	343	15.0	H_2	100
C_4H_4S	3000	457	20.0	H_2	100
	2500	171	7.5	33CO-67H_2	97

[a] Deviations from 100% are considered within precision of experiments.

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Table II. Determination of Sulfur Content of Liquids

Solvent	Sample	Sulfur Compound	Sulfur	Found[a]
	Ml.		*Micrograms/ml.*	%
No. 30 white oil	25	COS	13.7	92, 92
	25	CH_3SH	14.2	96
	25	C_4H_4S	110	102, 97
Benzene	5	CS_2	640	98, 101, 99
	2	C_4H_4S	8.3	99, 98, 100

[a] Deviations from 100% are considered within precision of experiments.

because the sulfur content dropped fairly rapidly with time when stored either in a water-sealed holder or in a metal cylinder.

Carbonyl sulfide is most readily converted to hydrogen sulfide. As shown in Table I, conversion is completed at 900° C. even in the absence of a catalyst. In the presence of alumina a temperature of 500° C. is sufficiently high to assure complete conversion of carbonyl sulfide and carbon disulfide, provided oxygen is absent from the gas. (Oxygen is automatically converted to water by the catalyst when operated at 900° C.) However, even in the absence of oxygen, thiophene and mercaptans are not completely converted to hydrogen sulfide at 500° C.

Examples of sulfur determinations in liquids are shown in Table II. In the case of the white oil solutions the oil was not vaporized over the catalyst; instead, the volatile sulfur was removed by stripping. Some of the sulfur solutions were unstable, as evidenced by a decrease in volatile sulfur with time. The benzene solutions were completely vaporized and passed over freshly regenerated catalyst. The carbon formation from a single analysis was not sufficient to interfere with the analysis.

ADSORPTION OF SULFUR ON CATALYST

Even at 900° C. some sulfur is retained by the alumina catalyst. The amount retained represents an equilibrium value for a given concentration and does not affect conversion. In analyzing a continuous gas sample of fairly uniform composition it is therefore sufficient to operate the catalyst until equilibrium is established (0.5 hour), after which a sample can be analyzed without danger of error due to adsorption. In case sulfur is to be determined in a batch sample of liquid or in a gas of varying composition, the catalyst must be completely purged with hydrogen before starting the analysis and again after all the sample has been treated. With 6 ml. of 8 to 14-mesh alumina at 900° C., complete desulfurization of the catalyst is achieved by passing 28 liters of pure hydrogen over it.

GENERAL APPLICATION

The analytical technique can be applied to a wide variety of gases and liquids. Successful analyses have been carried out on such gases as hydrogen, carbon monoxide, methane, ethylene, nitrogen, and coke-oven gas, and such liquids as methanol, benzene, cyclohexane, tetralin, and white oil. No tests have been made in which the hydrogen content of the vapors passing over the hydrogenation catalyst was less than 30%. The presence of water vapor is not necessary but it is helpful in some cases in reducing carbon deposition. Carbon dioxide does not interfere, provided it is not present in quantities sufficient to neutralize the caustic scrubbing solution. Unsaturated compounds, either initially present or formed over the catalyst, do not interfere. Oils and tars which collected in the scrubber may cause trouble if present in quantity. Should this occur, the scrubber solution can be acidified and the hydrogen sulfide stripped out and reabsorbed. Oxygen in concentrations up to 1% in the gas stream has been found harmless.

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Identification of Sulfur Compounds in Gas Mixtures

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A sensitive method for identification and quantitative determination of sulfur compounds present in gas mixtures depends on differences in solubility of the sulfur compounds in an inert solvent. A complete analysis usually involves both an absorption and a stripping run in a multiple-plate saturator. The total sulfur content of the gas leaving the saturator is plotted against gas volume. A stepwise curve results, wherein the gas volume at which the sulfur concentration changes abruptly is characteristic of a specific sulfur compound and the magnitude of the change is a measure of the concentration of that compound. As yet an exhaustive evaluation of the method to determine its accuracy has not been made, but identification runs on partially purified manufactured gas and coke-oven gas are presented, the results of which are in general agreement with previous industrial experience.

THE problem of completely removing sulfur compounds from gases is frequently encountered, particularly in catalytic processes. The most suitable means for effecting sulfur removal depends on the types present. Hydrogen sulfide is the most common form of sulfur encountered, but it is usually accompanied by relatively small concentrations of such organic sulfur compounds as carbonyl sulfide, carbon disulfide, and mercaptans. A new method has been developed for identifying and determining the concentration of these organic sulfur compounds when present in concentrations of a few parts per million. The technique is based on differences in solubility in an inert solvent used in conjunction with a highly sensitive analytical method (*1*, *2*). It is applicable not only to sulfur compounds but to any other class of compounds for which a sufficiently sensitive analytical method is available.

The accuracy and reliability of this method of identification have not been demonstrated on gases of known composition. Because of time limitations, the plant tests were made after only qualitative evaluation of the method on known gas mixtures. However, the results obtained on partially purified manufactured gas and coke-oven gas are in sufficient agreement with previous experience in the industry (*4*) to justify presentation at this time.

THEORY

The successful method herein presented is based on the following theory:

Henry's law generally applies to the solubility of gases in inert liquid at low concentrations. This law states that at equilibrium

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the concentration of a gas which will dissolve in a solvent is directly proportional to the partial pressure of the gas over the solvent.

$$p = H_T x$$

where p = partial pressure of solute over solution
H_T = proportionality constant at temperature T
x = mole fraction of solute in solvent

In the present application the sulfur compounds studied follow closely Raoult's law, which is a special case of Henry's law in which the proportionality constant is equal to the vapor pressure of the pure solute:

$$p = p_T x \quad (1)$$

where p_T = vapor pressure of pure solute at temperature T

Since the partial pressure of a component in a gas mixture is equal to the product of its mole fraction and the total pressure,

$$p = Py \quad (2)$$

where P = total pressure
y = mole fraction of solute in gas phase

Combining Equations 1 and 2, at equilibrium one mole of solvent will contain

$$x = \frac{Py}{p_T} \text{ moles of solute} \quad (3)$$

Let n equal the number of moles of gas of composition, y, containing this number $\frac{Py}{p_T}$, of moles of solute.

Then

$$ny = \frac{Py}{p_T} \text{ moles of solute in } n \text{ moles of gas} \quad (4)$$

or

$$n = \frac{P}{p_T} \text{ moles of gas} \quad (5)$$

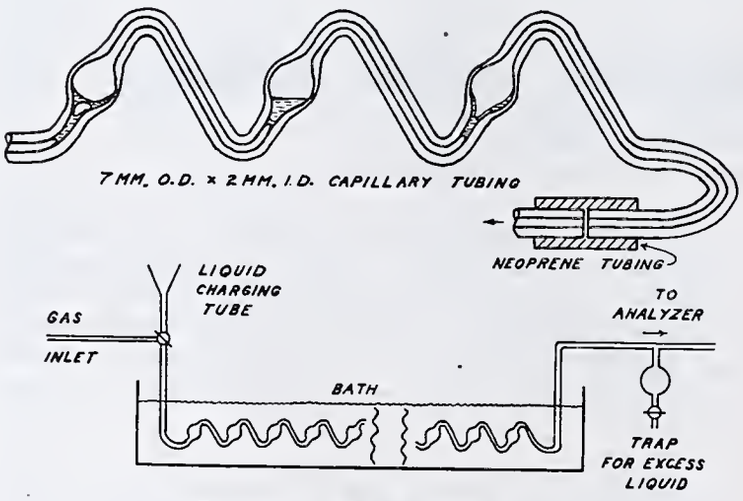

Figure 1. Diagram of Saturator

Thus the quantity of gas, n, which contains as much solute as one mole of liquid in equilibrium with it is dependent only on the ratio of the total pressure to the solubility coefficient or in this case the vapor pressure of the pure solute at the temperature in question. On the basis of a material balance, n is therefore the minimum quantity of the gas sample which could be used to saturate one mole of liquid with the solute and is a unique function of the solubility of this solute in the liquid. Hence an experimental determination of n reveals the solubility (or vapor pressure) of the solute. In the case of organic sulfur compounds this is usually sufficient to identify them. The relationships are the same, regardless of whether a liquid is being saturated by absorption of a component from a gas, or a gas is being saturated by stripping a component from a liquid.

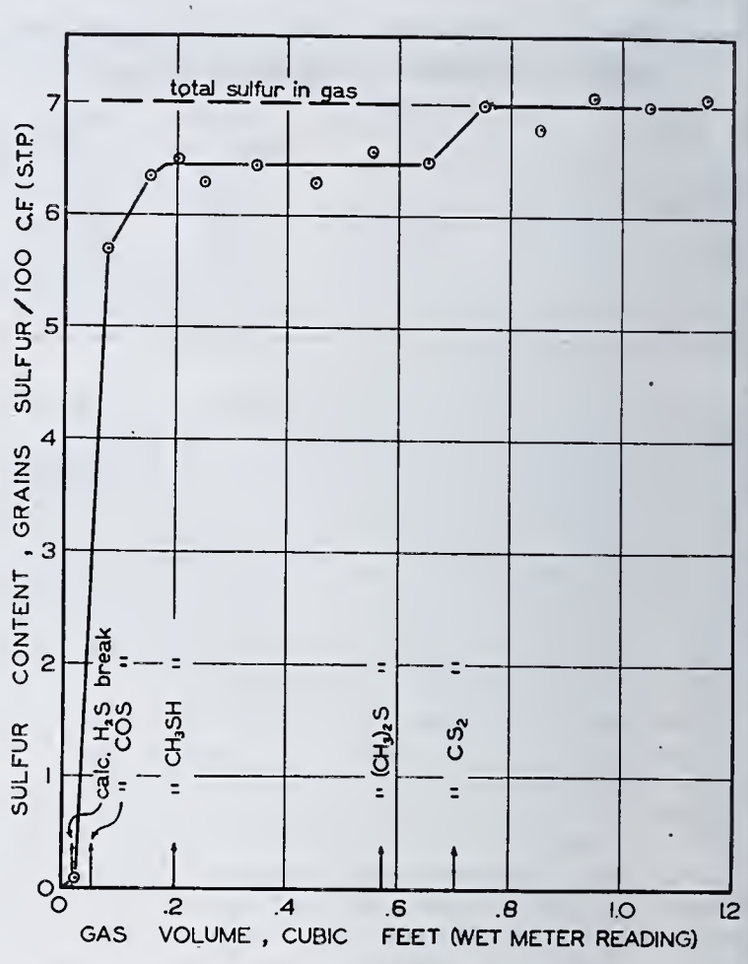

Figure 2. Composition of Blow Run Gas, Absorption Run

To determine these minimum gas quantities experimentally, it is necessary to have an efficient apparatus for contacting the liquid and gas, so that when absorbing the sulfur from a gas at least 80 to 90% of the liquid in the saturator can be saturated with the sulfur compound at the inlet gas composition before any of that compound appears in the exit gas, or when stripping sulfur from a liquid 80 to 90% of the saturated liquid will be stripped of a given sulfur compound before the sulfur concentration in the exit gas drops below the original saturation value. Such an apparatus is described below.

EQUIPMENT

A highly efficient saturator was constructed from twenty 60-cm. (2-foot) lengths of 2 × 7 mm. capillary tubing containing a total of 200 enlargements as illustrated in Figure 1. The sections are connected by butt joints with neoprene tubing. The scrubbing liquid, No. 30 white oil obtained from L. Sonneborn Sons, Inc., is introduced at the inlet end of the saturator (through the liquid charging tube) and distributed by passing a stream of hydrogen through the saturator. A very small quantity of liquid is retained in each enlargement, thereby providing 200 static pools of solvent. The entire unit is immersed in a liquid bath thermostated to ±0.5° C. A trap is provided at the exit end to catch any excess liquid carried out of the saturator.

The exit gases were analyzed by converting the sulfur compounds to hydrogen sulfide over alumina at 900° C. followed by spectrophotometric determination (*1*, *2*).

SAMPLE PROCEDURE AND CALCULATION FOR AN ABSORPTION RUN

The saturator was charged with 48 ml. of sulfur-free No. 30 white oil. The oil was distributed through the cells by means of cylinder hydrogen and the temperature adjusted to 14° C. Blow run gas from the generators was sampled after hydrogen sulfide removal in a Thylox unit by filling a stainless steel cylinder under pressure, and the analysis was started immediately. The gas was passed through the saturator at a rate of 0.005 cubic foot per minute and led directly to the analyzer previously described (*2*); total sulfur content was determined at frequent intervals. The

Table I. Vapor Pressure of Common Sulfur Compounds, Atmospheres

Compound	Temperature, ° C. −20°	−10°	0°	+10°	+20°	+30°	+40°	Reference
Hydrogen sulfide	5.39	7.53	10.2	13.6	17.7	22.6	28.3	(3)
Carbonyl sulfide	3	4.3	6	8.2	11	14	18	(3)
Methyl mercaptan	0.33	0.52	0.78	1.16	1.68	2.36	3.22	(3)
Ethyl mercaptan	0.095	0.158	0.251	0.398	0.602	0.890	1.284	(9)
Dimethyl sulfide	0.082	0.138	0.226	0.362	0.552	0.812	1.15	(5)
Carbon disulfide	0.061	0.101	0.132	0.263	0.392	0.550	0.816	(5)
Thiophene	0.008	0.016	0.029	0.050	0.082	0.13	0.20	(7)

volume of treated gas was plotted against its sulfur content (Figure 2). The total sulfur content of the unscrubbed gas was also determined and is shown by the dotted line.

Identification of the sulfur compounds present depends on the volume of gas corresponding to each "break" in the curve. For convenience in calculating the break volume, the vapor pressures of several common organic sulfur compounds have been collected in Table I. These values have been interpolated, extrapolated, or calculated where necessary to put the data in the form presented. The calculated break volumes for possible sulfur compounds are indicated by the arrows on Figure 2. When an increase in the sulfur content of the gas leaving the saturator occurs at a volume corresponding to the calculated break volume for one of the sulfur compounds it indicates the presence of that sulfur compound and the magnitude of the increase in sulfur content gives the concentration of that compound in the gas.

The break volume for carbon disulfide, for example, is calculated as follows:

$$n = \frac{P}{p_T} \tag{5}$$

p_T = 0.308 atmosphere for CS_2 at 14° C.
P = 1 atmosphere

giving n = 3.25 moles of gas to saturate 1 mole of oil with carbon disulfide.

But 48 ml. of oil were used (density 0.77, average molecular weight 154); so the amount of oil was $\frac{48 \times 0.77}{154 \times 454} = 0.000529$ pound mole. The amount of gas required to saturate the oil in the absorber is therefore $3.25 \times 0.000529 = 0.00172$ pound mole of gas. Under the wet meter conditions this gives a calculated break volume of $0.00172 \times 359 \times \frac{295}{273} \times \frac{760}{741} + 0.02$ cubic foot of free space in absorber = 0.70 cubic foot. The presence of carbon disulfide in the gas is shown in Figure 2 by the sudden rise in total sulfur at approximately the predicted 0.70 cubic foot break point. The amount of carbon disulfide is represented by the increase in sulfur corresponding to the break, or 0.6 grain of sulfur per 100 cubic feet (1 grain per 100 cubic feet = 22.9 micrograms per liter) at standard temperature and pressure.

The similarity in vapor pressure of hydrogen sulfide and carbonyl sulfide makes it difficult to distinguish between their break points. Hydrogen sulfide was therefore determined independently (1 grain of sulfur per 100 cubic feet) by absorption in cadmium acetate, made acid with acetic acid, followed by iodometric titration (8). Since the analysis of the gas at the end of the absorption run was 7 ± 0.3 grains of sulfur per 100 cubic feet, it was not possible to detect the presence of small concentrations of any sulfur compounds less volatile than carbon disulfide. However, by making a stripping run it was possible to determine the presence of any less volatile sulfur compounds because as the sulfur concentration in the gas drops below 1 grain per 100 cubic feet, the precision of the analysis becomes better than ±0.1 grain per 100 cubic feet as illustrated below.

EXAMPLE OF STRIPPING RUN

A 100-ml. sample of white oil was saturated with the same gas in the preceding example by bubbling the gas through the oil at a rate of 70 cubic feet per hour for 2 hours at −5° C. in a flask fitted with a sintered-glass sparger. About 50 ml. of this oil were transferred into the saturator and distributed by means of a slow stream of hydrogen, avoiding all contact with air. The saturated oil was stripped at 19.4° C. by bubbling a stream of hydrogen through it at a rate of 0.007 cubic foot per minute. The exit gas was analyzed for total sulfur and the sulfur content plotted against gas volume as before.

The break points for the different sulfur compounds were calculated exactly as in the preceding example. Because of the difference in temperature for the saturation and stripping, the concentration of sulfur in the absorber of gas did not correspond to that in the original sample. The correction was made by means of Raoult's law, as shown in the following example for carbon disulfide.

$$Py = xp_T \tag{3}$$

In this case,

P = 1
p_T = 0.416 atmosphere at 19.4° C.
p_T = 0.135 atmosphere at −5° C.

giving $y_{19.4°} = 0.416\,x$

and $y_{-5°} = 0.135\,x$

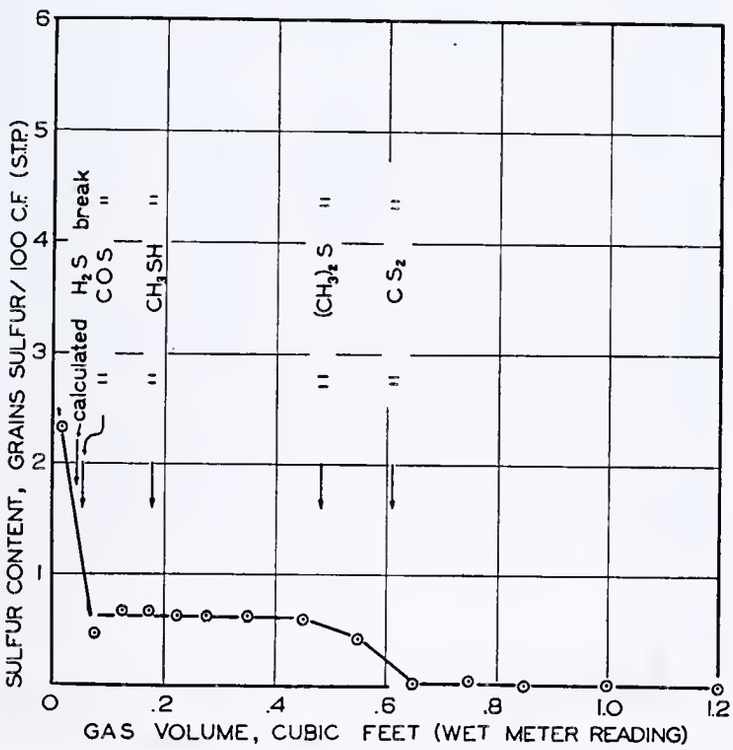

Figure 3. Composition of Blow Run Gas, Stripping Run

Since x, the concentration of gas in the solvent, is not changed when the saturated oil is warmed before stripping, it can be eliminated from the two equations to give

$$y_{-5°} = 0.33\,y_{19.4°}$$

Consequently, the concentration of carbon disulfide found in the stripping hydrogen must be multiplied by 0.33 to give the corresponding concentration in the original gas. A similar correction was made for each component present.

The volume of gas is shown plotted against sulfur content in Figure 3. For convenience in comparing with the absorption run on the same gas (Figure 2), the sulfur concentrations were corrected for temperature before plotting. As may be seen from the curve, most of the hydrogen sulfide and carbonyl sulfide was

stripped from the oil before good total sulfur analyses were obtained. It is for the less volatile sulfur compounds that the stripping run is most accurate. The absorption and stripping runs therefore supplement each other and a complete analysis usually requires both determinations.

Figure 4 is included to illustrate the behavior of a gas more concentrated in sulfur. In this case the gas is coke-oven gas partially purified by scrubbing with water, light oil, and ammonium carbonate solution. This preliminary treatment removed essentially all of the hydrogen sulfide and high-boiling sulfur compounds, leaving primarily carbon disulfide and a little carbonyl sulfide.

Table II. Sulfur Content of Plant Gases

Gas Analyzed	Grains of Sulfur per 100 Cubic Feet (S.T.P.) H_2S	COS	CH_3SH	CS_2	Higher boiling	Total
Coke-oven gas after removal of benzene, NH_3, H_2S, CO_2	0	0.7	0.8	46.5	0.5	48.5
Water gas from coke[a]						
Blue gas	0.5	3.6	0.25	0.35	0.02	4.7
Blow run gas	1.0	5.5	0	0.6	0.02	7.1

[a] After partial H_2S removal.

TECHNIQUE AND SOURCES OF ERROR

Certain organic sulfur compounds have been demonstrated (*2*) to be relatively unstable even in an inert solvent such as white oil. Exposure to oxygen or to metal walls can also affect the sulfur content of a gas or solution. For this reason analyses are completed as rapidly as possible and the procedures for sampling are arranged to minimize such losses. The equipment itself is of glass and quartz with neoprene-connected butt joints where required.

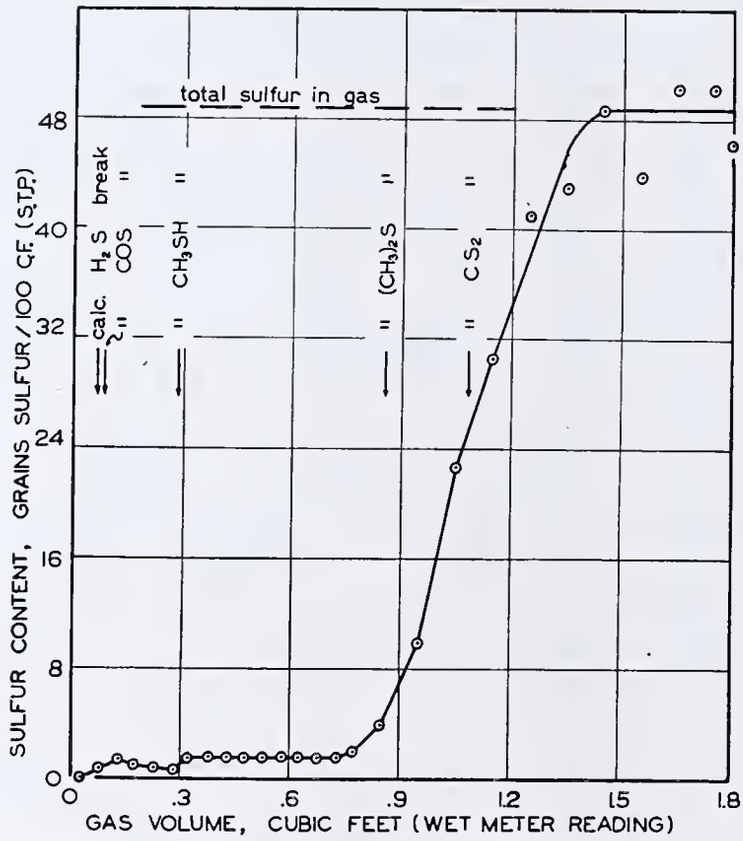

Figure 4. Composition of Coke-Oven Gas after Preliminary Scrubbing, Absorption Run

White oil was selected as the scrubbing liquid because it combines to an unusual degree the desirable properties of inertness, low volatility, and low viscosity. Other solvents can be used, provided they do not react with the sulfur compounds and are not volatilized to any large extent during the analysis. A more viscous solvent might require more plates in the saturator in order to give satisfactorily sharp breaks. The greater the number of plates the sharper the breaks but also the more solvent holdup, and hence the larger gas samples required for an analysis.

Since the volume of gas required for an identification run is directly proportional to the solubility of the sulfur compounds and the volume of solvent used, it is possible to separate the break points of two sulfur compounds of similar solubilities by increasing the quantity of solvent or lowering the temperature of the solvent to increase the solubility of the sulfur compounds.

The absorption of appreciable quantities of any component of the gas being analyzed will complicate the determination, since the net effect is to change the quantity and quality of the solvent medium. Analysis for the sulfur compounds present in a "wet" natural gas would offer this type of difficulty, since it might contain about 100 grains of gasoline per cubic foot of gas. Corrections for this type of interference can be made by measuring the increase in solvent volume and determining the solubility of the sulfur compounds in the resultant solvent.

Usually both an absorption and a stripping run are required for a complete analysis of a gas. If desired, the stripping run can be performed directly on the oil saturated in the absorption run. However, the length of time required for complete saturation may lead to errors arising from instability of the solution. The saturating technique described using a simple scrubbing bottle has the dual advantage of rapid saturation and convenient use of a low absorption temperature. Low-temperature saturation followed by higher temperature stripping increases the sulfur concentration in the stripping gas and therefore the accuracy of the determination.

The organic sulfur is converted to hydrogen sulfide for analysis as previously described (*2*), except that only 1 ml. instead of 6 ml. of alumina catalyst is employed. The smaller quantity of catalyst minimizes the adsorption of sulfur and hence gives a quicker response to changes in composition. The hydrogen sulfide may be determined by any sufficiently sensitive procedure, such as the spectrophotometric method (*1*) or the lead acetate–impregnated tape technique of Moses and Jilk (*6*). The sensitivity of the analyses for hydrogen sulfide is of paramount importance since, as can be seen from Figures 2, 3, and 4, it is necessary to obtain gas analyses at frequent intervals in order to detect sharp break points.

RESULTS

The results obtained with this method on three common commercial gases are summarized in Table II.

In coke-oven gas the major impurity remaining after the usual procedures for by-product recovery is carbon disulfide, although significant amounts of carbonyl sulfide, methyl mercaptan, and high-boiling sulfur compounds are also present. In water gas, on the other hand, carbonyl sulfide is the major impurity. Similar analyses following attempted purification are especially useful in revealing which types of compounds escape treatment.

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Evaluation of Laboratory Distillation Apparatus

Improved Oldershaw Glass Bubble Plate Columns, Automatic Still Heads, and Accessories

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Novel test methods have been developed for evaluating laboratory distillation apparatus under conditions approximating actual operation rather than under ideal conditions for maximum separation. In particular, the effect of reflux ratio on efficiency and separation has been studied. Test methods for evaluating the other principal characteristics of distillation apparatus are also described in detail. The Oldershaw glass bubble plate column has been improved and studied, using the test methods described. The column has been found highly satisfactory for analytical distillations of hydrocarbon mixtures because of uniformity of its operating characteristics. Automatic still heads and other accessory equipment have been developed which contribute to the uniformity of the performance of the column.

ANALYTICAL distillations are of increasing importance as a control and specification test for petroleum products and as a general research tool. In consequence, the comparative performance of the several types of distillation apparatus in general use is of considerable interest. However, data obtained for the operating variables of any given distillation apparatus may depend to a large degree upon the methods of measurement used. While Ward (*10*) has discussed in a comprehensive manner the interrelation of the principal variables in column performance, there is no generally accepted system of test methods for experimentally evaluating the significant variables of distillation apparatus performance. The usual practice has been to evaluate distillation apparatus under idealized conditions of total reflux, long equilibration time, etc. However, the extrapolation of such data to actual operation at finite reflux ratios may lead to questionable results in the case of small-scale laboratory columns. It has been found for such columns that actual separations obtainable at finite reflux ratios are generally appreciably lower than would be predicted from column efficiencies measured at total reflux.

A series of test methods has been devised for measuring the principal variables affecting column operation under conditions approximating actual operation. In particular, test procedures have been devised for evaluating column efficiency at finite reflux ratios, for measuring feed rates to the column, operating holdup, static holdup, heat loss, pressure drop, and the actual reflux ratios provided by automatic still heads.

This article includes a detailed description of test methods and an example of their application to an improved form of the Oldershaw bubble plate column (*6*).

Among the columns in common use in various laboratories for analytical distillations are the glass helices packed column (*11*), the Podbielniak Heligrid (*7*), the Stedman screen cone column (*9*), and the Oldershaw bubble plate column (*6*). Preliminary comparisons made on these columns indicated that the Oldershaw column has exceptionally high throughput capacity and a low operating holdup per theoretical plate relative to other columns. These facts, together with the fact that the fractionating efficiency of the column is nearly independent of the throughput rate and shows no significant variation with time or from column to column, make the bubble plate column an excellent tool for analytical distillations, especially in commercial laboratories where high fractionating efficiency for a given length of column is a secondary consideration compared with reproducibility of results and length of distillation time. The bubble plate column as originally described by Oldershaw has been improved by making the plates more uniform and by increasing the number of holes per plate, thus materially increasing the throughput capacity of the column. Auxiliary equipment, such as automatic still heads, timers, and product receivers, has been developed which has contributed to the reproducibility of the data obtainable with the glass bubble plate column. (The columns and other equipment tested were manufactured by the Glass Engineering Laboratories, San Carlos, Calif.)

DEFINITIONS

Because of the appreciable heat loss in laboratory batch distillations, the standard nomenclature used in large-scale continuous distillation is not readily applicable. The following definitions of terms used in the present article are given to avoid possible ambiguities.

Feed Rate (F, ml. per hour). The rate of entry of vapor into the bottom of the column, in terms of the equivalent volume of liquid.

Overhead Rate (O, ml. per hour). The rate of removal of vapor from the top of the column, in terms of the equivalent volume of liquid. This is equal to the reflux rate plus the product rate.

Condensation Rate (C, ml. per hour). The condensation rate due to heat loss in the column. This is equal to the difference between the feed and overhead rates.

Product Rate (P, ml. per hour). The liquid distillate take-off rate.

Reflux Rate (R, ml. per hour). The rate of return of condensed overhead to the top of the column.

Reflux Ratio (R/P). The reflux rate divided by the product rate.

The following relations hold for a batch column operating at constant feed rate.

$$F = O + C$$

$$O = P + R$$

$$P = \frac{F - C}{\frac{R}{P} + 1}$$

Number of Theoretical Plates. The number of successive infinitesimal vaporizations at equilibrium required to give the measured separation. The qualification "infinitesimal" requires measurements to be made at total reflux (or to be corrected for the effect of reflux ratio as described by McCabe and Thiele, *5*).

Plate Equivalents. A quantity numerically equal to the number of theoretical plates operating at total reflux which would give the separation obtained when the column is operating at a finite reflux ratio.

CRITERIA AND TEST METHODS

The following characteristics of column performance were studied:

1. Fractionating efficiency and its variation with feed rate.
2. Actual separation obtained at various reflux ratios.
3. Flood point or feed capacity with liquids of various surface tensions.

4. Pressure drop of the column and its variation with feed rate using liquids of various surface tensions.
5. Operating holdup and its variation with feed rate.
6. Drainage or static holdup.
7. Accuracy of the reflux ratios provided by the two types of still heads and the variation of reflux ratio with overhead rate.
8. Condensation in the column due to heat loss through the Dewar jacket and variation of heat loss from column to column.

All efficiency measurements were made with the *n*-heptane–methylcyclohexane binary using the equilibrium composition of Beatty and Calingaert (*2*) and the refractive index data of Bromiley and Quiggle (*3*). The refractive indices of the test samples were measured with a Pulfrich refractometer with a precision of 0.0001 unit which corresponds to about 0.13 theoretical plate in the 0.3 to 0.4 mole per cent region of the McCabe-Thiele diagram for *n*-heptane–methylcyclohexane. The refractive indices obtained for the pure materials are compared with the values given by Bromiley and Quiggle (*3*) in Table I.

Table I. Refractive Indices of Materials Used for Column Testing

Material	Boiling Point, ° C.	Observed Refractive Index, n_D^{20}	Literature[a] Refractive Index, n_D^{20}
n-Heptane	98.4	1.3878	1.3877
Methylcyclohexane	100.8	1.4229	1.4231

[a] Bromiley and Quiggle (*3*).

In making measurements at total reflux, the samples of the distillate and the reflux to the flask were collected as described by Oldershaw (*6*) after equilibrating the column for 1.5 hours. In the efficiency measurements at finite reflux ratios, the distillate was continuously returned to the flask through a U-tube, as diagrammatically shown in Figure 1, thus operating the column essentially as a continuous still. The separation efficiencies at finite reflux ratios were calculated in terms of theoretical plates by the method of McCabe and Thiele (*5*) and as "plate equivalents" as suggested by Baker *et al.* (*1*) by using the McCabe and Thiele diagram with the same operating line as at total reflux.

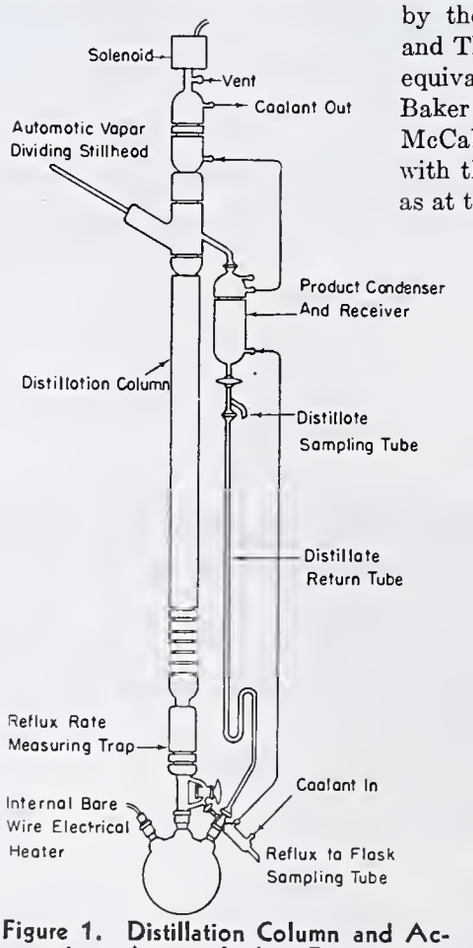

Figure 1. Distillation Column and Accessories Arranged for Fractionating Efficiency Tests

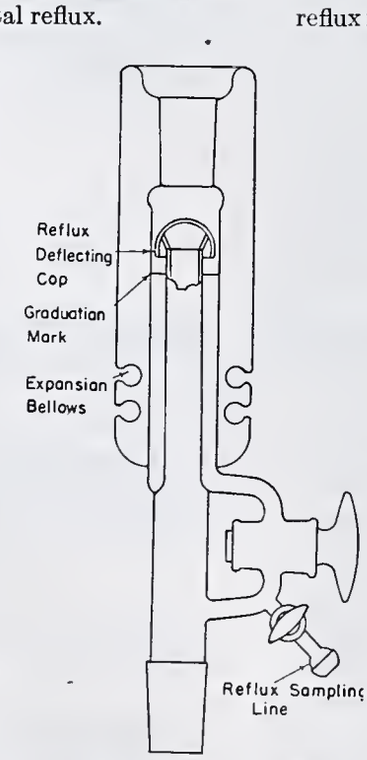

Figure 2. Trap for Measuring Feed Rate

The overhead rate could not be conveniently measured directly, since operating the column at total takeoff upsets the operating conditions because of the absence of reflux. Accordingly the column operating rates were determined in terms of the reflux to flask rate at total reflux (corresponding to the feed rate) which was measured with the reflux trap shown in Figure 2. The overhead rate differs from the feed rate by the condensation rate due to heat loss in the column and accordingly may be estimated from the feed rate if the heat loss of the column is known.

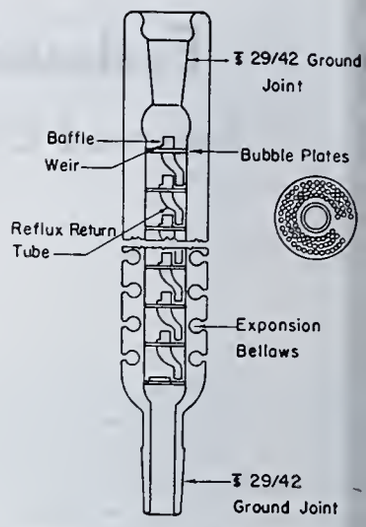

Figure 3. Improved Oldershaw Bubble Plate Column with Detail View of One Plate

The column heat loss was determined by measuring the reflux to flask when operating the column at a rate barely sufficient to produce reflux in the still-head condenser. The measured rate thus approximated the condensation rate of the column. It was established by other means that, as might be expected, the condensation rate of the column due to heat loss is substantially independent of the feed rate.

The pressure drop of the column was determined by measuring the difference in levels of the liquid in the U-tube connecting the flask and the still head. The feed capacity or flood point was determined by visual observation as the feed rate was slowly increased.

The operating holdup was measured by cutting off the current to the bare-wire immersion-heater and simultaneously closing the stopcock of the reflux trap, Figure 2. The liquid collecting in the trap was drained through a suitable cooler and was measured volumetrically.

The electrical timer was first calibrated by measurement of the off-on time ratios by means of a chronograph. The distillate rates delivered by the types of still heads at various nominal reflux ratios and overhead rates were then measured and the actual reflux ratios calculated. The overhead rates were measured without the column by mounting the still head directly on the reflux trap.

DISTILLATION APPARATUS

The items of distillation apparatus evaluated by the present test methods are described in detail below.

The improved Oldershaw column (Figure 3) consists of a series of perforated glass plates sealed into a tube. Each plate is equipped with a baffle to direct the flow of liquid, a weir to maintain the proper liquid level on the plate, and a reflux return tube. The inner diameter of the standard column is 26 to 28 mm. The holes in the plates are drilled with a red-hot tungsten wire of 0.89-mm. (0.035-inch) diameter. The number of holes per plate has been increased to 82 from the 42 described by Oldershaw (*6*). The ascending vapor stream flows through the holes in the plates and bubbles through the liquid reflux. The lowermost plate in the column serves as a small reservoir which is necessary to maintain a liquid seal for the drain tube from the first regular plate. The column is provided with an integral Dewar jacket with expansion bellows, permitting use of a 30-plate column to 250° C. The details of the method of construction have been described by Oldershaw (*6*).

The automatic vapor-dividing still head (Figure 4) includes a glass valve plunger which is arranged to direct the vapor stream selectively to the re-

Table II. Fractionating Efficiency and Operating Holdup of Improved Bubble Plate Column

Column dimensions: 27 to 28 mm. inside diameter, 30 actual plates, 80 holes per plate, 0.9-mm. diameter holes, 25-mm. spacing between plates)

Column Operating Rate		Reflux Ratio	Fractionating Efficiency			Operating Holdup	
Feed	Overhead	(to 1)	Theoretical plates	Plate efficiency	Plate equivalents	Total	Holdup quotient[a] ($\times 10^3$)
Ml./hour	*Ml./hour*			%		*Ml.*	
1500	1300	∞	17.2	57	..	47	1.82
2500	2300	∞	18.8	63	..	50.5	1.07
3000	2800	∞	19.4	65	..	53	0.91
3500	3300	∞	18.2	61	..	55.5	0.87
4000	3800	∞	16.7	56	..	59	0.88
1500	1300	27	17.1	57	12.6	47	1.83
2500	2300	27	17.4	58	12.7	50.5	1.06
3000	2800	27	18.0	60	12.7	53	0.98
3500	3300	27	17.0	57	12.6	55.5	0.93
4000	3800	27	16.6	55	12.3	59	0.89
3000	2800	64	18.2	61	15.5	53	0.97
3000	2800	42	18.0	60	14.4	53	0.98
3000	2800	13	14.9	50	9.1	53	1.18

[a] Ml. holdup per theoretical plate per ml. per hour feed.

lux condenser or to the product condenser. A direct current olenoid operates the plunger at timed intervals. The still-ead temperature is measured by a partial immersion thermom-ter provided with a standard-taper joint. The still head is ompletely Dewar-jacketed to improve the accuracy of the emperature measurements, to prevent heat losses from up-etting the accuracy of the reflux ratio, and to permit use of the till head with liquids that boil below room temperature. The ountercurrent relation of the vapor and reflux streams in the eflux condenser minimizes supercooling of the reflux. The reflux ondenser is preferably also Dewar-jacketed as shown to prevent rosting when low-boiling materials are distilled.

The alternative liquid-dividing still head (Figure 5) has a ilting funnel which receives the liquid stream from the reflux ondenser and normally returns it to the column. The funnel is ipped at timed intervals by a solenoid to deliver the liquid to the roduct receiver. The liquid-dividing still head is otherwise imilar to the vapor-dividing still head.

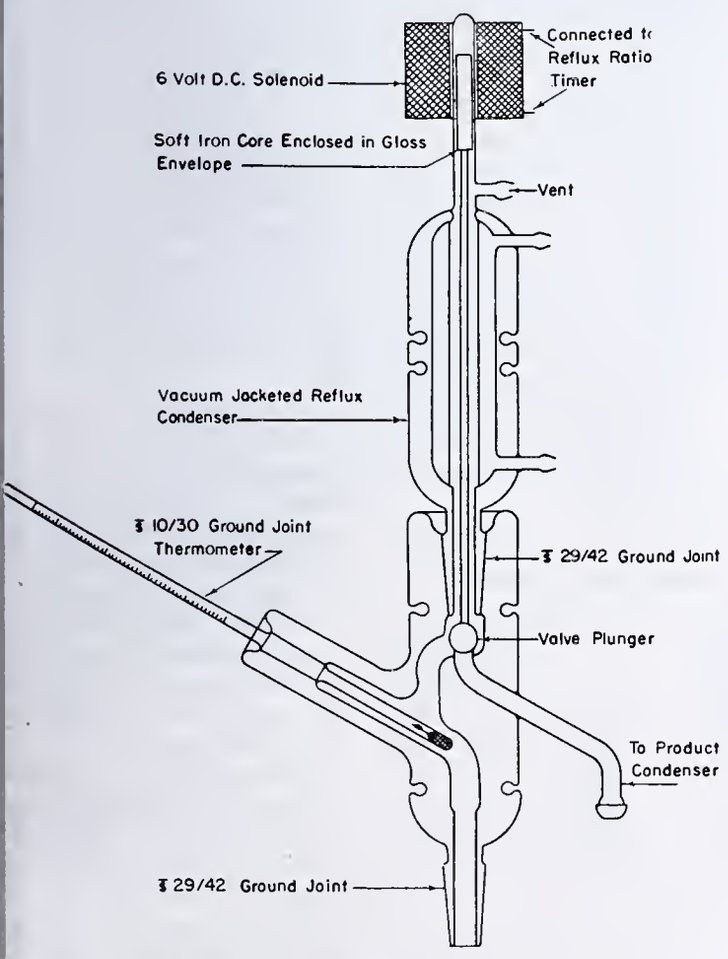

Figure 4. Automatic Vapor-Dividing Still Head

The reflux ratio controller consists of a time switch driven by a small synchronous motor and is of conventional design. It is adapted to deliver periodic currents of 3 seconds' duration and is adjustable to provide nominal reflux ratios in the range of 2/1 to 100/1.

The combined condenser and product receiver (Figure 6) consists of a liquid-jacketed graduated tube with a coiled vent line enclosed in the liquid jacket. For distillations of low-boiling liquids it is desirable to provide an external Dewar jacket as shown to prevent frosting.

A satisfactory flask and heater for analytical distillations should combine safety, low heat capacity, high potential heat input, and ability to distill the charge to negligible residue without excessive charring. No completely satisfactory flask and heater has been tested to date. For the purposes of column evaluation a 500-watt bare Chromel A wire immersion heater was used. It has the advantage of low heat capacity and immediate response to regulation.

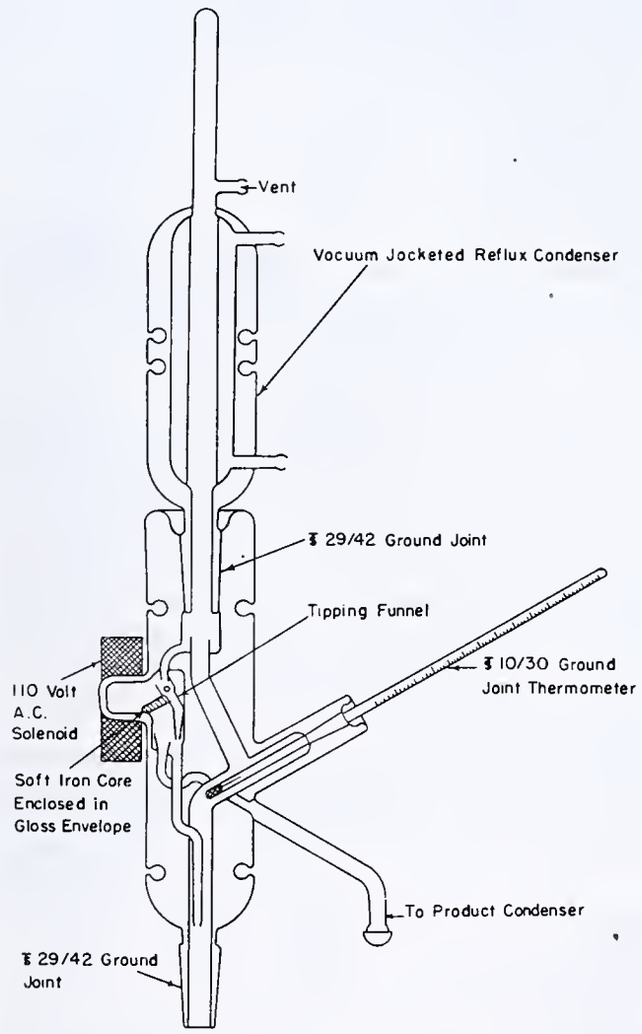

Figure 5. Automatic Liquid-Dividing Still Head

EXPERIMENTAL

Tests were conducted on a typical bubble plate column at infinite reflux ratio and at a nominal reflux ratio of 20 to 1 using various feed rates. Tests were also made at various reflux ratios using a constant feed rate of 3000 ml. per hour. The data given in Table II and presented graphically in Figures 7 and 8 show the effects of feed rate and reflux ratio upon column efficiency to be evident although not large. The fractionating efficiency has a peak value at a feed rate in the neighborhood of 3000 ml. per hour. However, optimum separation is obtained at a given product rate by operating the column at a somewhat higher feed rate and a higher reflux ratio. This may be seen from Figure 9 in which

Table III. Comparative Fractionating Efficiencies and Heat Losses of Sample Bubble Plate Columns

Column No.	Maximum Feed Rate	Fractionating Efficiency[a]		Condensation Rate of *n*-Heptane Due to Heat Loss
		Theoretical plates	Plate efficiency	
	Ml./hour		%	*Ml./hour*
1	5200	19.4	65	200
2	5150	19.1	64	260
3	5200	19.3	64	170
4	4730	19.9	66	180
5	4500	20.8	69	190
6	4250	19.8	66	230

[a] At 3000 ml. per hour feed and infinite reflux ratio.

separation expressed in plate equivalents is plotted against feed rate, the reflux ratio being varied to provide a constant product rate.

A number of bubble plate columns of identical dimensions were tested with respect to efficiency under similar conditions of overhead rate and reflux ratio. The data (Table III) indicate no appreciable difference in fractionating efficiency among the several columns.

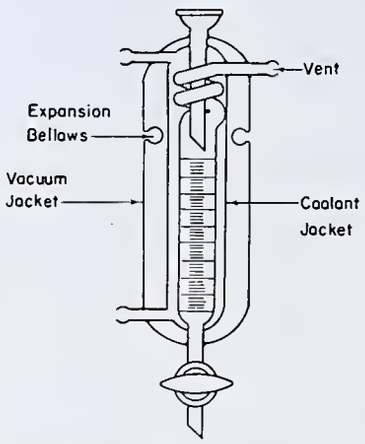

Figure 6. Combined Condenser and Product Receiver

The data on operating holdup at various feed rates and reflux ratios are given in Table II and shown in Figures 7 and 8. It will be seen that the holdup increases nearly linearly with feed rate and is practically independent of the reflux ratio. The holdup is also given in terms of operating holdup per theoretical plate per unit feed rate, an important quantity in column evaluation. Rose, Welshans, and Long (*8*) have shown that the sharpness of separation obtainable in a batch distillation is approximately a linear function of the ratio of charge to holdup. Thus, decreasing the holdup relative to feed rate enables a sharper separation to be obtained with the same number of theoretical plates and same total distillation time. Therefore gains in efficiency accompanied by increase in operating holdup as in the bubble plate column of Langdon and Tobin (*4*) may lead to an actua decrease in the sharpness of separation in a given batch dis tillation.

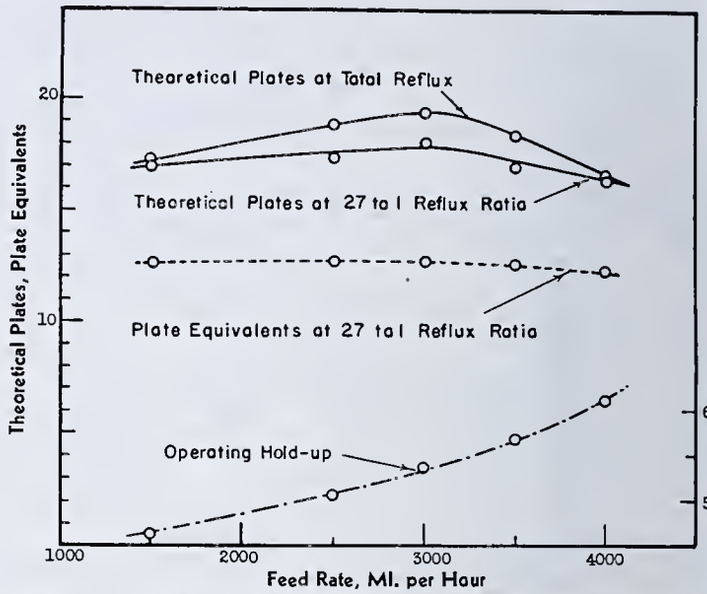

Figure 7. Influence of Feed Rate upon Efficiency, Separation, and Operating Holdup

30-actual-plate column

The residual drainage or static holdup of the column afte draining 10 minutes is 0.6 ml. total or 0.02 ml. per plate whe distilling *n*-heptane. Longer drainage time does not reduce th static holdup. The static holdup does not vary significantl with the viscosity in the case of light hydrocarbon distillates.

The pressure drop and feed capacity of the bubble plate colum are largely dependent upon the surface tension of the liquid bein distilled, because of the capillarity of the bubble plate holes The pressure drop of the column was measured at various rate using several different liquids. The relevant physical propertie of these liquids are summarized in Table IV. The data given i Table V indicate that the column is satisfactory for material such as hydrocarbons but is less suitable for liquids having hig surface tension, because of greatly reduced overhead capacit Small amounts of water in the material being distilled may rende the column inoperative. Accordingly, material which may con tain water should be dried with a suitable desiccant before distil ling. The pressure drop of the column at reduced still-head pres sures was also measured using a mixture of *n*-heptane and methyl cyclohexane. The data given in Table VI indicate a shar dropping off of overhead capacity with decreasing still-head pres sure. Thus, for high feed rates, the column is not useful at still head pressures much below 250 mm.

The maximum overhead capacity of a well-constructed bubbl plate column should be such as to permit a feed rate of 5000 ml per hour when a mixture of methylcyclohexane and *n*-heptane i refluxed. However, since th poorest bubble plate determine the maximum reflux rate, flood ing may occur at feed rate considerably below 5000 ml. pe hour. Columns having maxi mum feed rates in excess of 400 ml. per hour are ordinarily con sidered satisfactory.

Heat loss data for a numbe of typical bubble plate column of 30 actual plates are given i Table III. The condensatio rate due to heat loss is ver nearly independent of the fee rate for a material of given boil ing point and heat of vaporiza

Table IV. Physical Properties of Materials Used for Pressure Drop Measurements

Material	Boiling Point	Density		Surface Tension	
		At 20° C.	At b.p.	At 20° C.	At b.p.[a]
	° *C.*	*Grams/ml.*		*Dynes/cm.*	
n-Heptane	98.4	0.6837	0.6180	20.26	12.5
Methylcyclohexane	100.8	0.7691	0.6989	23.73	15.7
Benzene	80.1	0.8790	0.8153	29.02	21.3
Formic acid	100.8	1.220	1.170	37.6	29.0

[a] Calculated from values at lower temperatures using Eötvös equation.

Table V. Pressure Drop of Bubble Plate Column with Liquids of Various Surface Tensions

n-Heptane			Methylcyclohexane			Benzene			Formic Acid		
Feed rate	Pressure drop per actual plate			Pressure drop per actual plate			Pressure drop per actual plate			Pressure drop per actual plate	
Ml./hour	*Mm. Hg*	*Mm. liquid*[a]	*Ml./hour*	*Mm. Hg*	*Mm. liquid*[a]	*Ml./hour*	*Mm. Hg*	*Mm. liquid*[a]	*Ml./hour*	*Mm. Hg*	*Mm. liquid*[a]
1000	..	..	1000	..	..	1000	0.76	12.7	200	0.88	10.2
1500	0.50	11.1	1500	0.62	12.1	1500	0.82	13.6	400	0.95	11.0
2000	0.53	11.6	2000	0.63	12.3	2000	0.84	13.9	600	1.02	11.8
2500	0.55	12.0	2500	0.65	12.6	2500	0.87	14.4	800	1.09	12.7
3000	0.57	12.5	3000	0.67	13.0	3000	0.91	15.2	1000	1.18	13.7
3500	0.60	13.1	3500	0.71	13.8	3500	1.00	16.7	1125[b]	1.23	14.3
4000	0.64	14.1	4000	0.77	15.0	4000	1.11	18.5	...	..	..
4500	0.70	15.4	4500	0.85	16.5	4280[b]	1.17	19.5	...	..	..
5000	0.77	16.9	5000	0.93	18.1	...	..	..	...	..	..
5230[b]	0.80	17.6	5230[b]	0.97	18.9	...	..	..	...	..	..

[a] Mm. of refluxing liquid at boiling point.
[b] Flood point of column with given liquid.

Table VI. Pressure Drop of Bubble Plate Column at Various Still-Head Pressures

(Material refluxed: mixture of *n*-heptane and methylcyclohexane, surface tension at 100° C. = 13.2 dynes per cm.)

Feed Rate	Still-Head Pressure Drop per Actual Plate					
	760 Mm. Hg		500 Mm. Hg		250 Mm. Hg	
Ml./hour	Mm. Hg	Mm. liquid[a]	Mm. Hg	Mm. liquid[a]	Mm. Hg	Mm. liquid[a]
1500	..	..	..	..	0.668	13.1
2000	0.598	12.3	0.624	12.6	0.724	14.2
2500	0.612	12.6	0.649	13.1	0.852	16.7
3000	0.632	13.0	0.718	14.5	Floods	
3500	0.675	13.9	0.802	16.2		
4000	0.719	14.8	Floods			

[a] Based upon total pressure drop as measured at U-tube in distillate return tube (see Figure 1).

tion. The heat loss may vary considerably from column to column. A well-constructed vacuum jacket, however, should allow a condensation rate of only about 200 ml. per hour of *n*-heptane.

Two types of completely vacuum-jacketed automatic still heads have been used with the bubble plate column—i.e., the vapor-dividing and the liquid-dividing types (Figures 2 and 3). While both types are satisfactory with respect to providing accurately reproducible reflux ratios, the vapor-dividing type is believed to be preferable for the following reasons: A well-constructed vapor-dividing still head provides less product leakage at total reflux than the liquid-dividing type. The fragile moving parts are more easily removed and repaired in the vapor-dividing than in the liquid-dividing type. The vapor-dividing type tends to provide actual reflux ratios which are closer to the off-on ratio of the electrical timer than does the liquid-dividing type. The liquid-dividing type is disadvantageous in that it permits loss of reflux to the product take-off in the event of flooding of the still head. This is an important consideration if the still head is to be used in conjunction with a packed column, because proper technique calls for the preliminary flooding of the column to wet the column packing thoroughly before commencing distillation.

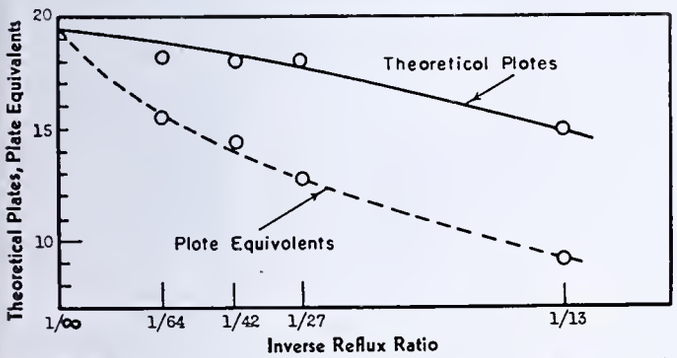

Figure 8. Effect of Reflux Ratio upon Efficiency and Separation

Feed rate, 3000 ml. per hour. 30-actual-plate column

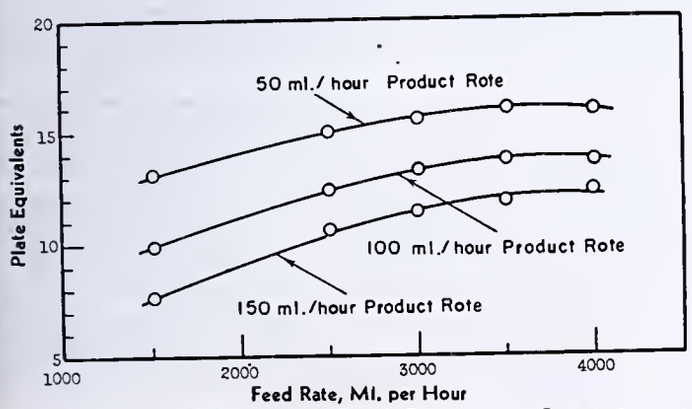

Figure 9. Influence of Feed Rate upon Separation at Constant Product Rate

Reflux ratio increased with increasing feed rate to provide constant product take-off rate. 30-actual-plate column

Data for the actual reflux ratios provided by particular still heads of the two types at various overhead rates and nominal reflux ratios are given in Tables VII and VIII. It will be noted that, in all cases, the actual reflux ratios provided by the still heads are higher than the off-on time ratios provided by the electrical timer. This can be compensated for by setting the timer to a ratio slightly lower than that which it is desired to maintain. The reflux ratio provided by either of the two types of still head appears to be nearly independent of the overhead rate.

The actual reflux ratio provided by a given still head will depend upon such considerations as distance of travel of the valve plunger or tipping funnel, adjustment of solenoid with respect to its armature, and amount of condensation produced in the lower portion of the still head by the supercooled reflux stream.

The present evaluation of the operating characteristics of the Oldershaw bubble column, the automatic still heads herein described, and other accessories shows the apparatus to be well suited to requirements of analytical distillations of hydrocarbons.

Table VII. Effect of Overhead Rate upon Actual Reflux Ratios of Automatic Still Heads of Two Types

(Electrical timer ratio: 24.1 to 1)

Overhead Rate, Ml./Hour	Actual Reflux Ratio (to 1)	
	Vapor-dividing still head	Liquid-dividing still head
2000	28.4	31.1
2500	27.8	30.0
3000	27.4	29.4
3500	27.0	29.1
4000	26.7	28.9

Table VIII. Actual Reflux Ratios Provided by Automatic Still Heads at Various Electrical Timer Ratios

(Overhead rate: 3500 ml. per hour)

Electrical Timer Ratio (to 1)	Actual Reflux Ratio (to 1)	
	Vapor-dividing still head	Liquid-dividing still head
3.7	4.3	4.1
6.3	7.3	8.0
11.6	13.0	14.0
24.1	26.9	28.9
43.3	42.2	50.0
66.5	63.8	77.3

ACKNOWLEDGMENTS

It is the authors' pleasant duty to acknowledge the suggestions and work of many members of the staff of Shell Development Company in the development of the present distillation apparatus and in particular to William B. Milligan who contributed the detail design of the automatic still heads. They are indebted to Fred D. Tuemmler and Louis Lykken for their advice and suggestions in the course of the experimental work.

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Determination of Sodium and Potassium in Silicates

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Upon the addition of a 20% solution of hydrogen chloride in anhydrous *n*-butyl alcohol (Willard and Smith reagent) to a butyl alcohol solution of the perchlorates of sodium and potassium, the two alkali metals are precipitated quantitatively as a mixture of sodium chloride, potassium chloride, and potassium perchlorate. This precipitation can be carried out in the presence of most elements commonly found or occasionally encountered in silicates and is proposed as basis of a new method for the determination of sodium and potassium.

WILLARD and Smith (*8*) introduced a solution of hydrogen chloride in anhydrous *n*-butyl alcohol as a reagent for the quantitative separation of sodium from lithium, and Kallmann (*4*), making use of the difference in solubility of the perchlorates and chlorides of the three alkali metals in butyl alcohol containing the same reagent, described a method for the quantitative separation of lithium from both sodium and potassium.

It was shown that lithium perchlorate and chloride are very soluble, that potassium is precipitated as a mixture of perchlorate and chloride, and that sodium perchlorate which is only sparingly soluble in butyl alcohol is quantitatively converted into the insoluble chloride.

As sodium and potassium are quantitatively precipitated by the solution of hydrogen chloride in butyl alcohol (Willard and Smith reagent), this solution suggested itself as the logical precipitant for sodium and potassium for their quantitative separation from the nonalkali constituents of silicates.

Preliminary work, recorded in Table I, indicated that the perchlorates of aluminum, calcium, magnesium, and iron which are all very soluble in butyl alcohol, are not precipitated by the Willard and Smith reagent. Manganese perchlorate, which decomposes upon heating and forms a residue in butyl alcohol, is soluble as chloride in butyl alcohol containing the Willard and Smith reagent.

When fuming large amounts of titanium salts with perchloric acid, some decomposition takes place. Any precipitate thus formed can be conveniently filtered off, however, after diluting with water. Since soluble titanium perchlorate is not precipitated by the Willard and Smith reagent, moderate amounts of this element do not interfere in the method suggested below.

More serious is the behavior of barium, strontium, and lead from silicates containing these elements in an acid-soluble form. The perchlorates of barium, strontium, and lead are very soluble in both water and butyl alcohol. Like sodium, they are precipitated, presumably quantitatively, by the Willard and Smith reagent. Certain precautions requiring hardly any additional work and obviating the difficulties just described, have been incorporated in the method described below.

About 2.7 kg. (6 pounds) of the Willard and Smith reagent, used in this investigation, were prepared by passing dry hydrogen chloride from a tank into a beaker containing 2.26 kg. (5 pounds) of anhydrous *n*-butyl alcohol. The beaker was externally cooled and the gassing interrupted several times, until the butyl alcohol was nearly saturated with hydrogen chloride at room temperature and showed a specific gravity of about 0.905, indicating an approximately 20% solution of hydrogen chloride in butyl alcohol.

PROCEDURE

DECOMPOSITION OF SAMPLE. Transfer 0.5 to 1.0 gram or more, depending on the alkali metal content, of the finely ground sample to a 50- to 100-ml. platinum dish, moisten with water, and add 20 ml. of 47% hydrofluoric acid and 15 ml. of 72% perchloric acid. Stir well with a platinum wire or the rubber end of a policeman and evaporate with moderate heat on a hot plate until copious white fumes of perchloric acid escape. Remove from the hot plate, wash down the sides of the dish with a little water, and again heat until fumes of perchloric acid escape freely and about half of the acid has been expelled. Do not fume to dryness, since this may cause thermal decomposition of perchlorates.

When cool, take up with 20 ml. of hot water and warm until all salts have dissolved. A residue at this stage denotes incomplete decomposition of the sample or presence of large amounts of titanium or of barium sulfate.

Filter through a small paper into a 250-ml. beaker and wash 8 to 10 times with hot water. If there is any doubt as to whether the sample was completely decomposed, ignite the paper in the original platinum dish, again treat with small portions of hydrofluoric and perchloric acids, evaporate to near dryness, take up with water, and filter into the main solution.

Evaporate on a hot plate to fumes of perchloric acid, finally expelling the excess of acid. To prevent decomposition of perchlorates, particularly of aluminum perchlorate, which would render subsequent separations more difficult, moderate heat only should be used, and expulsion of the perchloric acid must not be carried too far. The resulting perchlorates should not be bone-dry, but a small amount of perchloric acid, indicated by the escape of white fumes, should be left.

A limited number of silicates particularly highly aluminous minerals, yield incompletely to the action of hydrofluoric acid. Others may be high in titanium, barium, or lead. For such samples and additional complex inorganic compounds an alternative way of decomposition is desirable.

Table I. Solubility of Perchlorates in *n*-Butyl Alcohol and in Butyl Alcohol Containing Willard and Smith Reagent

Element	Weight Taken, Mg.	Solubility of Perchlorates in Butyl Alcohol	Effect of Willard and Smith Reagent
Sodium	100 ± 5	Clear solution	White precipitate
	500 ± 5	White residue	White precipitate
Potassium	100 ± 5	White residue	No apparent change
Lithium	300 ± 10	Clear solution	Clear solution
Aluminum	100 ± 5	Clear solution	Clear solution
	500 ± 5	Clear solution	Clear solution
Calcium	100 ± 5	Clear solution	Clear solution
	500 ± 5	Clear solution	Clear solution
Magnesium	200 ± 10	Clear solution	Clear solution
Iron	100 ± 5	Clear, brown solution	Clear, pale yellow solution
Manganese	100 ± 5	Brown residue	Clear solution
Titanium	10 ± 1	Clear solution	Clear solution
	100 ± 10	Cloudiness	Cloudiness
Barium	100 ± 5	Clear solution	White precipitate
Strontium	100 ± 10	Clear solution	White precipitate
Lead	100 ± 5	Clear solution	White precipitate
Zirconium	50 ± 5	Clear solution	Clear solution
Vanadium	50 ± 10	Clear solution	Clear soluton
Chromium	10 ± 2	Clear solution	Clear solution

The chlorides of the elements listed were fumed to near dryness with an excess of perchloric acid. The perchlorates were heated to boiling with 25 ml. of butyl alcohol and the solution was examined for insoluble compounds; 11 ml. of the Willard and Smith reagent were then added, the boiling was continued for one minute, and after cooling to room temperature, the effect of the precipitant was noted.

The only choice in such cases is the J. L. Smith method (*7*), details of which can be found in analytical textbooks. Applying the proposed method directly to the aqueous extract from the original fusion, containing the chlorides of the alkali metals along with considerable calcium, some of the more serious objections to the J. L. Smith method are overcome by adding 10 ml. of perchloric acid to the water extract from the Smith fusion, and evaporating on a hot plate until copious fumes of perchloric acid escape, and finally until almost dry.

PRECIPITATION OF SODIUM AND POTASSIUM. To the cold perchlorates add 25 ml. of anhydrous *n*-butyl alcohol and heat the solution to boiling. (A precipitate at this stage consists of potas-

sium perchlorate, and with a little experience, it is not difficult to estimate the approximate potassium content of the sample, a fact which is most valuable to the analyst who intends to confine his work to the determination of only one of the two alkali metals. The potassium perchlorate can be filtered off on a Gooch crucible, converted to the chloride by ignition, dissolved in water, and finally determined as potassium perchlorate.)

Add dropwise to the boiling solution 3 ml. of the Willard and Smith reagent and then 8 ml. more. (A white precipitate formed at this point denotes the presence of sodium which can be observed even in the presence of large amounts of potassium.)

Allow to simmer for 3 minutes, cool to room temperature, decant the supernatant liquid into a dry Gooch crucible, transfer the bulk of the precipitate onto the crucible without policing the beaker, and wash 8 to 10 times with 1- to 2-ml. portions of a 6 to 7% solution of hydrogen chloride in butyl alcohol, made by diluting 40 ml. of the Willard and Smith reagent with 100 ml. of butyl alcohol. Discard filtrate and washings, unless wanted for the determination of a nonalkali constituent of the sample.

Separation of Sodium and Potassium. Dry the beaker and Gooch crucible for about 15 minutes on a hot plate or in an electric drying oven. Then dissolve the precipitate on the Gooch, and also any particles adhering to the sides of the beaker, in a minimum amount of hot water. For this purpose it is desirable to arrange the filtering apparatus so that the filtrate can be directly caught in a 250-ml. beaker.

Add 5 ml. of perchloric acid and evaporate on a hot plate nearly to dryness. Since small amounts of nonalkali elements may have escaped the preceding separation, it is not advisable as suggested in the Smith and Ross procedure (*6*) to expel all perchloric acid at this stage. This is done, however, during the reprecipitation of the potassium perchlorate.

Cool, add 15 to 20 ml., depending on the alkali metal content, of a mixture of equal parts per volume of butyl alcohol and ethyl acetate, digest near boiling for 2 to 3 minutes, cool, and decant the supernatant liquid into a dry but unweighed Gooch crucible. Again the filtering apparatus should be arranged so that the filtrate can be caught directly in a small beaker. Wash the potassium perchlorate three times by decantation with 3-ml. portions of the mixed solvent. Reserve filtrate and washings.

Dissolve the residue in the crucible in a minimum amount of hot water, catching the solution in the original beaker, add 1 ml. of perchloric acid, evaporate to dryness, and expel any acid condensed on the side walls of the beaker by brushing with a free flame. Cool, dissolve salts in 3 to 5 ml. of water, and again evaporate to dryness. Cool, add 10 to 15 ml. of the mixed solvent, digest near boiling as before, cool to room temperature, and filter through the original crucible which in the meantime has been dried, ignited, cooled, and weighed. Transfer the residue to the Gooch crucible, police the beaker, and wash the crucible 8 to 10 times with 1-ml. portions of the mixed solvent. Reserve filtrate and washings which have been caught in the original beaker containing the bulk of the sodium. Dry the precipitating beaker for a few minutes on a hot plate and brush any unremoved particles of potassium perchlorate into the Gooch crucible. Dry the crucible for 1 hour at 110° C. and finally, while covered with a watch glass, for 15 minutes at 350° C. Cool and weigh as potassium perchlorate.

Determination of Sodium. The subsequent treatment of the butyl alcohol–ethyl acetate solution containing the sodium depends largely on the amount of sodium present.

If the Willard and Smith reagent, as precipitant for sodium and potassium in their separation from the nonalkali constituents of the sample, caused little or no apparent precipitation of sodium, the logical choice should be one of the two triple acetate methods (*1*, *2*).

Evaporate the combined filtrates and washings, after diluting with one third their volume of water, on the water bath in such a way as to avoid condensation on the upper part of the beaker.

When completely dry, add 10 ml. of water, 1 ml. of nitric acid, and 1 ml. of sulfuric acid and evaporate on a hot plate to strong fumes of sulfuric acid. This treatment is usually sufficient to destroy any brown coloration due to organic matter. If not, a few drops of nitric acid should be added and the fuming resumed. Finally, fume to complete dryness, dissolve salts in a few milliliters of hot water, filter off any insolubles if necessary, evaporate to 1 ml., cool, and precipitate the sodium with triple acetate reagent, directions for which can be found in analytical textbooks.

If the Willard and Smith reagent caused considerable precipitation of sodium chloride, sodium should finally be precipitated and determined as the chloride.

Table II. Separation of Sodium and Potassium from a Number of Elements

Element or Compound	Weight Taken	NaCl Used	KCl Used	Regular Method NaCl found	Regular Method KCl found	Rapid Routine Method, Combined Chlorides Found
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg*	*Mg.*
....	...	50.0	50.0	50.4	59.2	....
....	...	10.0	100.0	9.7[a]	100.9	....
....	...	100.0	100.0	....	....	199.2
Al	150	100.0	100.0	99.8	101.1	....
	300	100.0	100.0	....	....	200.5
Ca	50	100.0	50.0	100.8	50.4	....
	300	3.0	100.0	3.3[a]	101.3	....
Mg	100	50.0	50.0	.....	....	100.8
	300	100.0	100.0	100.2	100.4	....
Fe	20	50.0	50.0	.....	....	99.9
Mn	20	5.0	50.0	5.0[a]	49.7	....
Ti	20	50.0	50.0	49.3	49.6	....
Ba	25	50.0	50.0	49.5[b]	50.6	....
	20	50.0	50.0	51.0[c]	49.8	....
Pb	20	50.0	50.0	50.3[b]	50.7	....
	20	50.0	50.0	51.8[c]	49.7	....
As	10	50.0	50.0	50.6	49.8	....
V	10	50.0	50.0	.....	....	100.4
H_3PO_4	32	10.0	50.0	9.6	50.8	....
	32	100.0	100.0	.....	....	201.2
H_3BO_3	40	50.0	50.0	.....	....	99.3
	200	50.0	50.0	.....	....	100.7
CHOHCOOH	250	50.0	50.0	48.7	48.9	....
CHOHCOOH	1000	10.0	50.0	7.8[a]	48.2	....
Mixture of						
Al	150	50.0	50.0	.....	....	100.2
Ca	50					
Mg	50	6.0	50.0	6.6[a]	50.4	....
Fe	5					
Ti	10	100.0	100.0	100.8	101.0	....
H_3PO_4	32					

[a] Sodium determined as triple acetate.
[b] Sodium determined as chloride after removal of Ba or Pb as carbonates.
[c] Sodium determined as sulfate after removal of Ba or Pb as sulfates.

Evaporate the combined filtrates and washings from the potassium determination, after diluting with water, in the same way as for the triple acetate method. When completely dry, add 10 ml. of water and 3 ml. of perchloric acid and evaporate slowly, with a cover glass on, to strong fumes of perchloric acid. Increase the heat until all organic matter is destroyed. Finally evaporate to near dryness.

Add 15 to 20 ml. of butyl alcohol and heat the solution to boiling. Add 7 or 9 ml., respectively, of the Willard and Smith reagent, the first 2 ml. dropwise, allow to simmer for one minute to facilitate coagulation of the sodium chloride, cool to room temperature, collect the precipitate on a dry Gooch crucible, and wash 5 times with 1- to 2-ml. portions of the 6 to 7% solution of hydrogen chloride in butyl alcohol.

Dry the crucible for half an hour at 110° C., then ignite for 5 minutes in a muffle at 600° C. Cool in a desiccator, then weigh the crucible plus sodium chloride. Dissolve the precipitate in hot water, collecting and reserving the washings in a small beaker, dry the crucible for one hour at 110° C., cool, and weigh. The loss in weight represents sodium chloride.

The sodium chloride solution can be tested for possible presence of barium or lead by one of two procedures.

1. Add 1 gram of ammonium carbonate, heat to boiling, and allow to stand for one hour. The solution should remain clear. If it contains any precipitate, filter it off, evaporate the filtrate to dryness, transfer with hot water to a weighed platinum dish, add a few drops of hydrochloric acid, evaporate to dryness, heat as usual to expel any ammonium chloride, and weigh as pure sodium chloride.
2. Precipitate any barium or lead present by adding 1 ml. of sulfuric acid and determine the sodium in the filtrate as sodium sulfate.

Rapid Routine Method. Collect the mixture of alkali chlorides and potassium perchlorate, obtained as described above, on a dry Gooch crucible, policing the beaker in which the precipitation was carried out. Special attention should be paid to the amount of potassium perchlorate present, prior to the addition of the Willard and Smith reagent, and also to the amount of sodium chloride precipitated.

Dry the crucible for one hour at 110° C., then increase the heat of the muffle gradually to 650° C., at which temperature the crucible is kept for half an hour, while covered with a watch glass or platinum cover. Cool and weigh, and dissolve chlorides of sodium and potassium in hot water, receiving filtrate and washings

directly in a small beaker. Dry the crucible for one hour at 110° C., cool, and weigh. The loss in weight represents the combined chlorides of sodium and potassium.

Depending on the relative amounts of sodium and potassium present, based largely on observations made during the precipitation with the Willard and Smith reagent, precipitate either the sodium as triple acetate or the potassium as perchlorate or chloroplatinate and calculate the other alkali metal from the weight of the combined chlorides.

Acid-soluble barium or lead salts would accompany the sodium and potassium. In such cases, the aqueous solution of the combined chlorides should be treated with a small amount of ammonium carbonate and the chlorides of sodium and potassium recovered in the filtrate of the lead or barium carbonate.

CORROBORATION AND VERIFICATION. Both methods described above were applied to the quantitative separation and determination of sodium and potassium in the presence of elements which form the main constituents of silicates or may occasionally be encountered.

All nonalkali elements and compounds used in these tests were specially prepared or tested to be alkali-free. In order to duplicate as much as possible all operations that a sample of silicate has to go through, the hydrochloric or nitric acid solution of these elements and the aqueous solution of acids were evaporated to dryness in a platinum dish. Hydrofluoric and perchloric acids were then added and the methods, described in the preceding paragraphs, applied. The results, presented in Table II, show that in the proposed method direct precipitation of sodium and potassium in the presence of large amounts of other elements is possible.

An attractive feature is the fact that the method provides at an early stage of the analysis definite indications as to the relative amounts of sodium and potassium present.

In contrast to the J. L. Smith method (*7*), no solid reagents are used if the sample can be decomposed by acid treatment. No ammonium salts are used. They would interfere because of the small solubility of ammonium perchlorate in butyl alcohol containing the Willard and Smith reagent and in the mixture of butyl alcohol and ethyl acetate.

Another advantage lies in the removal of lithium at an early stage of the analysis, thus obviating one of the major objections inherent in the Smith and Berzelius methods.

While magnesium may interfere in the Smith method, and definitely does interfere in the Berzelius method (*3*), and causes the use of 8-hydroxyquinoline in the more recent method of Marvin and Woodlaver (*5*), it causes no difficulty in the proposed method.

Moderate amounts of phosphoric, boric, and even tartaric acids do not interfere. The effect of the sulfate ion was investigated (Table III).

Any sulfate ion remaining after the fuming with perchloric acid will cause formation of sodium sulfate, and to a lesser degree, of potassium sulfate, both insoluble in butyl alcohol. The data in Table III indicate that the Willard and Smith reagent causes considerable conversion of these sulfates into the equally insoluble and noninterfering chlorides:

$$Na_2SO_4 + 2HCl \longrightarrow 2NaCl + H_2SO_4$$
$$K_2SO_4 + 2HCl \longrightarrow 2KCl + H_2SO_4$$

As much as 15 mg. of sulfur in form of sulfates apparently is quantitatively converted into chlorides and does not interfere. For larger quantities of sulfates, the writer would suggest either reprecipitation of the mixture of alkali chlorides and potassium perchlorate, or, preferably, treatment of the aqueous solution of these alkali salts with a few drops of barium chloride, after converting them to the chlorides by ignition, and simultaneous removal of the excess reagent with ammonium carbonate.

The method discussed above was applied in the determination of sodium and potassium in a number of representative standard samples of silicates and related products (Table IV).

Table III. Effect of Sulfate Ion

NaCl Used	KCl Used	0.1 *N* H_2SO_4 Used	Equivalent to Sulfur	Sulfur Found: Combined chlorides	Sulfur Found: NaCl	Sulfur Found: $KClO_4$ + KCl	Sulfur Found: 6% solution of HCl in butanol
Mg.	*Mg.*	*Ml.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
50	..	1	1.6	..	..	..	1.4
50	..	4	6.4	..	0.1	..	6.6
100	..	8	12.8	..	0.2	..	12.2
100	..	10	16.0	..	0.4	..	15.5
100	..	15	24.0	..	0.8	..	22.0
..	50	1	1.6	..	..	..	0.8
..	100	8	12.8	..	..	0.4	12.8
..	100	15	24.0	..	..	0.6	23.8
100	100	5	8.0	0.3	..	..	8.1
100	100	15	24.0	1.2	..	..	23.2

Tbe solution of sodium and/or potassium chloride to which varying amounts of 0.1 *N* sulfuric acid were added, was fumed to near dryness with perchloric acid and treated witb butyl alcohol and the Willard and Smitb reagent, and the sulfate ion determined in the resulting fractions.

Table IV. Determination of Sodium and Potassium in Standard Samples

B. of S. Sample No.	Type of Sample	Certificate Value: Weight of Sample	Certificate Value: Combined cblorides	Certificate Value: Na_2O	Certificate Value: K_2O	By Analysis: Combined cblorides	By Analysis: Na_2O	By Analysis: K_2O
		Grams	*Gram*	%	%	*Gram*	%	%
70	K_2O = feldspar	1.0000	0.2439	2.38	12.58	0.2433[a]	2.27[b]	12.66
		1.0000	0.2439	2.38	12.58	0.2440[a]	....	...
		1.0000	0.2439	2.38	12.58	.....	2.35	12.68
		1.0000	0.2439	2.38	12.58	.....	2.41	12.53
		0.5000[c]	0.1220	2.38	12.58	0.1224[a]	....	...
99	Na_2O = feldspar	1.0000	0.2088	10.73	0.41	0.2100[a]	10.82[b]	0.38
		1.0000	0.2088	10.73	0.41	.....	10.75	0.44
		1.0000	0.2088	10.73	0.41	.....	10.68	0.37
		0.5000[c]	0.1044	10.73	0.41	0.1049[a]	....	...
		0.5000	0.1044	10.73	0.41	.....	10.74	0.44
91	Opal glass	1.0000	0.2112	8.48	3.25	0.2118[a]	....	...
		1.0000	0.2112	8.48	3.25	.....	8.50	3.25
		1.0000	0.2112	8.48	3.25	.....	8.52	3.32
		0.5000[c]	0.1056	8.48	3.25	0.1065[a]	....	...
		0.5000[c]	0.1056	8.48	3.25	.....	8.44	3.22
80	Soda-lime glass	1.0000	0.3145	16.65	0.04	0.3149[a]	16.70	0.02
		1.0000	0.3145	16.65	0.04	.....	16.65	0.00
		0.5000[c]	0.1572	16.65	0.04	0.1570[a]	....	...
98	Plastic clay	1.0000	0.0554	0.28	3.17	0.0557[a]	....	...
		1.0000	0.0554	0.28	3.17	0.0560[a]	0.26[d]	3.21
		1.0000	0.0554	0.28	3.17	.....	0.30[d]	3.15
		1.0000	0.0554	0.28	3.17	.....	0.30[d]	3.14
		0.5000[c]	0.0277	0.28	3.17	0.0285[a]	0.28[d]	3.17
89	Pb-Ba glass	1.0000	0.2405	5.70	8.40	0.2415[a,e]	....	...
		1.0000	0.2405	5.70	8.40	.....	5.72[f]	8.42
		1.0000	0.2405	5.70	8.40	.....	5.81[g]	8.40
		0.5000[c]	0.1203	5.70	8.40	.....	5.73	8.46
		0.5000[c]	0.1203	5.70	8.40	0.1200[a,e]	5.67[b]	8.48
1[a]	Limestone	1.0000[i]	0.0186	0.39	0.71	.....	0.43[d]	0.74
		2.0000[i]	0.0372	0.39	0.71	.....	0.36	0.72

[a] Combined chlorides by rapid routine metbod.
[b] Na_2O calculated by difference from weight of combined chlorides.
[c] J. L. Smith fusion applied.
[d] Sodium determined as triple acetate.
[e] Pb and Ba previously removed with ammonium carbonate.
[f] Sodium determined as cbloride after removal of Pb and Ba witb $(NH_4)_2CO_3$.
[g] Sodium determined as sulfate.
[i] Sample decomposed with $HClO_4$ in covered dish, prior to addition of HF.

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Polarographic Determination of Tin in Foods and Biological Materials

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A rapid and precise polarographic method for the determination of tin in foods and biological materials has been developed, which is applicable to the determination of tin in concentrations as low as 0.5 part per million. The accuracy is of the order of ±5% for concentrations in excess of 10 parts per million and somewhat less for smaller amounts. It has been demonstrated that moderate amounts of iron, copper, bismuth, cadmium, mercury, cobalt, and zinc may be present without interference. Lead leads to high results and a procedure in case lead is present is outlined.

THE accurate determination of traces of tin in foods is of considerable interest and has many limitations. The method adopted by the Association of Official Agricultural Chemists (*2*) has been widely used for many years, but while it is very reliable it is subject to a number of disadvantages. In order to obtain satisfactory precision it is necessary to handle large samples, usually 50 to 100 grams, and the wet digestion of such large samples is time-consuming and requires large quantities of oxidizing acids. The hydrogen sulfide separation is also objectionable, from the standpoint of both the toxicity of the hydrogen sulfide and the tedious filtration of the slimy precipitate.

The polarographic procedure described permits the determination of tin on a much smaller sample with the elimination of the sulfide separation. Greater sensitivity is obtained and the precision in the lower range is greatly enhanced.

At the time this study was instigated the behavior of solutions of tin at the dropping mercury electrode was not completely understood. Lingane (*6*) found that stannous tin in tartrate solutions yielded a well-defined anodic wave due to the oxidation of stannous to stannic tin and a well-defined cathodic wave corresponding to the reduction of stannous tin. No wave was obtained for stannic tin. The same author (*4*) reported a similar composite wave for stannous tin in sodium hydroxide solution and a rather poorly defined double wave for the reduction of stannic tin in hydrochloric acid solution. Smrz (*8*) obtained well-defined waves for the reduction of stannous tin in hydrochloric acid. Lingane and Scott (*3*) reported that stannous tin was not reducible in perchloric acid, but that well-defined waves were obtained on addition of chloride ion. A similar behavior was found for stannic tin in perchloric acid. In the presence of added chloride a double wave was obtained. Alimarin (*1*) reported an application of the polarograph to the determination on tin in ores in which stannic tin was reduced in a supporting electrolyte of hydrochloric acid and sulfuric acid.

A very complete discussion of the theoretical considerations of the polarography of stannic tin in halide solutions was presented by Lingane (*7*) while this investigation was in progress. Lingane, working with pure solutions, recommended a supporting electrolyte of ammonium chloride in hydrochloric acid with a trace of gelatin to suppress maxima. After the present paper had been submitted for publication, a polarographic procedure for the determination of tin and lead in copper base alloys was described (*5*).

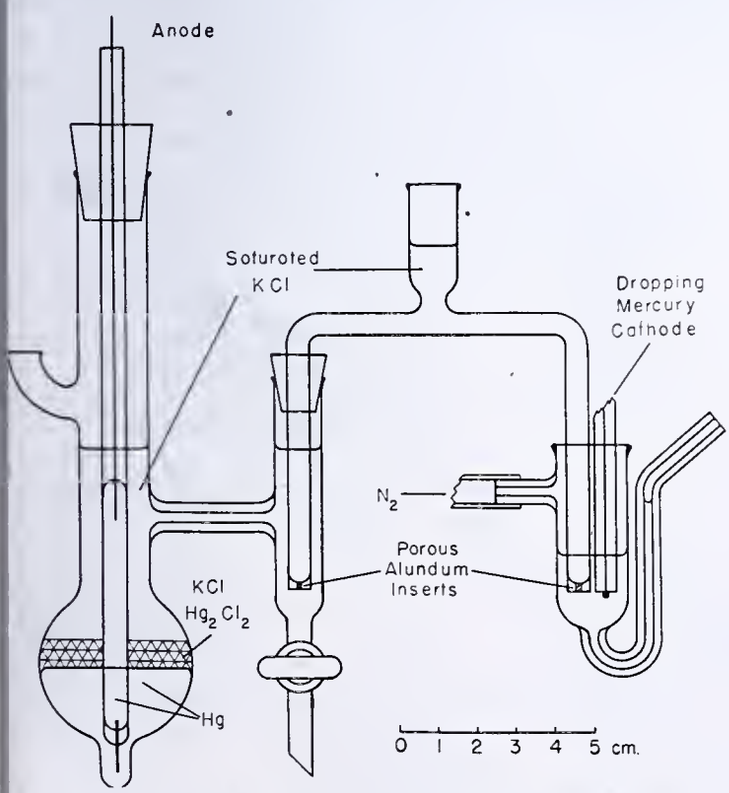

Figure 1. Calomel Electrode and Electrolysis Cell Assembly

APPARATUS

The usual polarograph electrolysis cell of the simple Heyrovský type is not applicable to the determination of tin, since the anode pool effects some reduction of stannic tin. For this reason it is necessary to use an external reference anode. In the work under discussion a saturated calomel cell was used, the construction of which is shown in detail in Figure 1.

The cell is made of heavy-walled Pyrex and is designed to minimize concentration polarization by virtue of the relatively large area of mercury exposed. Connection to the electrolysis vessel is made through an inverted U-tube salt bridge filled with a saturated solution of potassium chloride. Small pieces of porous Alundum are fused into the tips of the bridge. The electrical resistance of the bridge is low, and the potassium chloride diffuses at such a low rate that the sample is not measurably diluted.

The electrolysis vessel (Figure 1) is of very simple construction. Dissolved oxygen is swept out of the solution by passing a current of nitrogen through the capillary tube. When this flow is stopped and diverted to the side arm, a blanketing atmosphere of nitrogen is maintained over the solution in the cell. A manifold system permits the simultaneous deaeration of several samples, preventing any loss of time at this point of the procedure. The cell described not only permits the increased sensitivity associated with the use of a relatively small volume of solution (3 to 4 ml.), but in conjunction with the use of the external electrode permits a maximum economy of mercury. Only a few tenths of a gram are used per determination and this is completely recoverable.

A Heyrovský-type polarograph manufactured by E. H. Sargent & Co. was used throughout the course of this investigation. The full-scale galvanometer sensitivity was 0.0028 microampere per millimeter. A 60- to 65-mm. length of marine barometer tubing was used as the cathode capillary and under an applied pressure of 75 cm. of mercury the drop time was approximately 3.3 seconds with the capillary immersed in distilled water. Under these conditions 1.94 mg. of mercury were delivered per second. Although it would be desirable to work under constant-temperature conditions, the measurements reported were made at room temperature and are subject to a small error which under normal operating conditions probably does not exceed 5%.

EXPERIMENTAL

Although very satisfactory anodic waves can be obtained for the oxidation of stannous tin (*3, 4*), it was felt that considerable

difficulty would be associated with obtaining and maintaining the tin in a completely reduced state, and that it would be preferable to work with solutions of stannic tin. In preliminary experiments it was shown that well-defined waves suitable for analytical purposes could be obtained in a medium of dilute sulfuric acid containing a soluble chloride such as ammonium chloride, sodium chloride, potassium chloride, or hydrochloric acid and a trace of certain organic substances such as gelatin, methyl red, or cresol red which act as maximum suppressors. It was shown that the reduction of tin occurred in two very definite steps, first a reduction of the tetravalent tin, supposedly existing as the anion of the acid H_2SnCl_6 to the divalent state; then, in the second step, the subsequent reduction to metallic tin. With increasing concentrations of chloride ion the two waves became more widely separated and more sharply defined. The second wave marking the reduction of divalent tin to metallic tin approximated the form of a typical reversible reaction, while the shape of the first wave indicated that the current in this step of the reduction was limited by factors other than diffusion, probably by the dissociation of the aquo-stannic ion. In concentrated chloride solutions the increase in diffusion current due to the second step of the reduction is approximately equal to one half of the total diffusion current. It was further demonstrated that the second portion of the reduction wave was well suited to analytical measurement, since the diffusion current could readily be measured and was directly proportional to tin concentration. These experimental results and conclusions are in substantial agreement with those of Lingane (*7*).

Some difficulty was encountered in attempts to apply the procedure outlined above to the determination of tin in the sulfuric acid residues resulting from the acid digestion of food products. Results in most instances were completely satisfactory but extremely abnormal curves were obtained for occasional samples. The current in these instances would increase rapidly and almost linearly with increasing applied voltage. Similar curves were obtained on solutions yielding normal curves to which were added small amounts of nitric acid containing oxides of nitrogen. The same behavior could also be induced by passing a small amount of nitric oxide into the solution prior to electrolysis. It was inferred that the occasional abnormal curves were due to incomplete decomposition and removal of nitrosyl sulfuric acid formed during the digestion step. It was observed that when solutions giving these abnormal curves were boiled vigorously and again diluted to volume the polarograms were usually normal and recovery determinations were fairly satisfactory. Even when this procedure was followed, an occasional failure was noted, especially during the analysis of such products as evaporated milk, sardines, and shrimp.

In order to eliminate entirely the occurrence of these troublesome failures, methods of separation and concentration were studied. It was shown that tin could be completely precipitated as the hydroxide and, in the presence of added aluminum, the precipitated hydroxides could readily be separated by centrifuging. The supernatant liquid was removed by decantation, and the precipitate dissolved in hydrochloric acid and diluted to volume with a saturated solution of ammonium chloride. The solutions so prepared produced uniformly consistent and well-defined curves entirely free of the abnormality noted previously. In addition, the separation step permitted a reduction in volume with an accompanying increase in sensitivity.

DETAILS OF PROCEDURE

Reagents. A. Ammonium chloride. Saturated solution in distilled water.

B. Hydrochloric acid (1 + 1). Dilute C.P. acid with an equal volume of distilled water.

C. Aluminum chloride. Dissolve 4.65 grams of aluminum chloride nonahydrate in distilled water and dilute to 250 ml. 1 ml = 2 mg. of Al.

D. Standard tin solution. Dissolve 0.500 gram of pure tin in 250 ml. of hydrochloric acid and dilute to 500 ml. with distilled water. For use dilute 25 ml. to 250 ml. with dilute hydrochloric acid (reagent B). 1 ml. = 0.1 mg. of Sn.

E. Cresol red. Saturated solution of *o*-cresolsulfonphthalein in distilled water.

Preparation of Samples. Transfer a 5- to 10-gram sample of the food product to a 250-ml. Erlenmeyer flask and add 10 to 15 ml. of nitric acid and 2 to 3 ml. of sulfuric acid. Heat slowly to begin the oxidation. As charring occurs add nitric acid in small increments. When sulfur trioxide fumes are evolved with no further charring or darkening, add 1 ml. of perchloric acid and continue heating until the perchloric acid is volatilized and sulfuric acid is refluxing freely on the walls of the flask. Allow to cool and add 10 to 15 ml. of distilled water.

Separation of Tin. Transfer the digested sample to a 50-ml. conical centrifuge tube. A long-taper tube accurately graduated at 10 ml. is most desirable. Rinse the flask with a small volume of water and finally dilute to approximately 20 ml. Add 1 ml. of aluminum chloride solution (reagent C) and one drop of methyl red indicator. Make alkaline with concentrated ammonium hydroxide and add 0.1 to 0.2 ml. in excess. A large excess of ammonium hydroxide is to be avoided, since tin and aluminum hydroxides become somewhat soluble. Centrifuge at high speed until the precipitate has been well packed in the tip of the tube. A trunnion-head centrifuge was found to be somewhat more satisfactory than a fixed-angle machine, since the precipitate is packed more firmly into the tip of the tube and the supernatant liquid may be poured off with less danger of disturbing the precipitate. Decant the clear supernatant liquid as completely as possible. To the residue in the tube add 2.5 ml. of hydrochloric acid (reagent B) and shake to effect solution. Add sufficient solution (reagent A) to bring the final volume to 10.0 ml.

Polarography. Transfer a 4- to 5-ml. portion of the prepared solution to a clean dry electrolysis cell and add one drop of cresol red solution (reagent E). Bubble nitrogen through the solution for 10 minutes, then divert the flow of gas to provide a blanketing flow over the surface of the solution. Record the polarogram between 0 and 0.8 volt at the highest galvanometer sensitivity which will permit the recording of the complete curve. Measure the height of the second tin wave in the manner illustrated in Figure 2 and calculate the tin content of the sample, using the appropriate sensitivity factor and the calibration constant obtained from standard samples.

Calibration. Transfer accurately measured aliquots of the standard tin solution (reagent D) to centrifuge tubes, dilute with distilled water, and precipitate the tin as directed above. Record the polarograms and measure the wave height, then plot

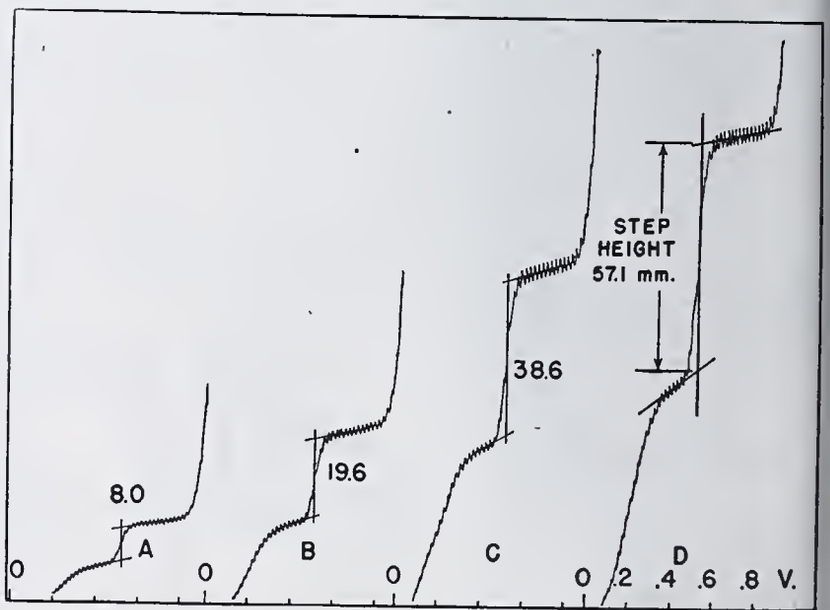

Figure 2. Polarographic Determination of Tin

In 1.5 *N* HCl and approximately 4 *N* NH_4Cl. Cresol red as maxima suppressant
A. 10 micrograms per ml.
B. 25 micrograms per ml.
C. 50 micrograms per ml.
D. 75 micrograms per ml.
Recorded at 1/20 full-scale sensitivity, 1 mm. = 0.056 microampere

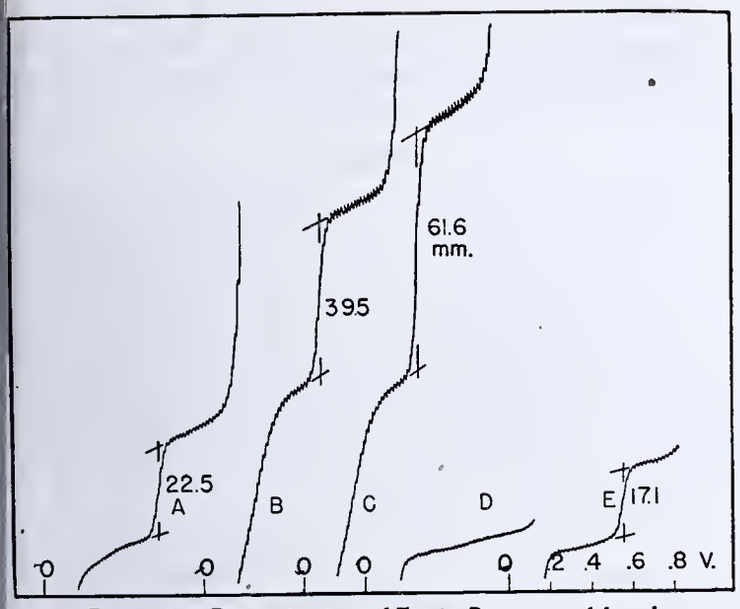

Figure 3. Determination of Tin in Presence of Lead

A. 200 micrograms of Pb in 10 ml. of acid medium
B. 200 micrograms of Sn in 10 ml. of acid medium
C. 200 micrograms of Pb + 200 micrograms of Sn in 10 ml. of acid medium
D. 200 micrograms of Sn in 13 ml. of alkaline medium
E. 200 micrograms of Sn + 200 micrograms of Pb in 13 ml. of alkaline medium
Recorded at 1/10 full-scale sensitivity, 1 mm. = 0.028 microampere

the wave height against tin concentration, multiplying the measured heights by the appropriate sensitivity factor. Draw a straight line through the points and from the slope of the line compute the conversion factor relating wave height to tin concentration. Each capillary must be calibrated, and additional standards should be run at intervals. With proper care a capillary will last almost indefinitely.

INTERFERING ELEMENTS

The behavior of a number of potential interfering elements was studied in order to determine their effect on the determination of tin by the method described. Some of these elements are eliminated to a certain extent in the ammonium hydroxide separation. It was demonstrated that iron, copper, bismuth, cadmium, mercury, antimony, nickel, cobalt, and zinc could be present in concentrations equal to or slightly greater than that of the tin without noticeable effect on the results. The presence of lead, however, may lead to some error, since it is precipitated with the tin and is reduced at a voltage too near that of the second tin wave to be resolved. The results of these determinations are shown in Table I.

Table I. Effect of Extraneous Elements on Recovery of Tin

Added Metal	Amount Added *Micrograms*	Tin Found (200 Micrograms Added) *Micrograms*
Fe	200	204
	500	200
	1000	200
Cu	200	200
	500	200
	1000	200
Bi	200	200
	500	200
	1000	200
Cd	200	200
	500	200
	1000	202
Hg	200	204
	500	202
	1000	202
Sb	200	200
	500	202
	1000	202
Ni	200	200
	500	202
	1000	202
Co	200	200
	500	200
	1000	202
Pb	200	324

Table II. Recovery of Tin in Presence of Lead by Modified Procedure

Amount of Lead Added *Micrograms*	Tin Found (400 Micrograms Added) *Micrograms*
0	400
200	405
500	391
1000	409

The potential error due to lead interference is not regarded as a serious disadvantage in the application of the method to the determination of tin in foods, since lead, if present, usually occurs in relatively minor concentrations. In the event that the presence of significant quantities of lead is suspected, it is possible to overcome the interference as outlined below. This modified procedure involves recording the polarogram on the solution in the usual manner, then making a portion of the acid solution strongly alkaline by the addition of a measured amount of ammonia and ammonium citrate, and recording a second polarogram. In the ammoniacal citrate solution the lead wave is well defined and the tin wave is completely suppressed.

Polarography of Tin in the Presence of Lead. Record the polarogram as usual on the ammonium chloride–hydrochloric acid solution and determine the combined wave height due to tin and lead. To a 5.0-ml. aliquot of the solution add 1.0 ml. of concentrated ammonium hydroxide and 0.5 ml. of ammonium citrate (50% w/v). Record the polarogram of this solution and measure the height of the lead wave. Prepare solutions containing no tin and varying amounts of lead as directed in the section on calibration. Precipitate the aluminum and lead and dissolve in the hydrochloric acid–ammonium chloride supporting electrolyte. Record the polarogram on the acid solution and on the ammoniacal citrate solution prepared as directed above. Measure the heights of the lead waves and plot the wave heights in the acid medium against those of the corresponding solutions in the alkaline medium. It is unnecessary to correct the diffusion current in the alkaline medium for the dilution, since the same dilution factor is introduced in both standards and unknowns. The slope of this line is determined by the dilution factor and by the relative diffusion rates of the lead ions in the two media and hence should remain constant as long as the composition of the two solutions is unchanged. Using this curve, convert the height of the lead wave obtained for the unknown in alkaline medium to the equivalent wave height for the same amount of lead in acid medium. Deduct this computed wave height from the combined wave height of the tin and lead in acid medium to obtain the net diffusion current due to tin.

Figure 3 shows a series of polarograms illustrating the method of correcting for lead interference. Polarogram D shows a very small inflection due to the trace of lead in the reagents used. For very precise determinations of minute concentrations added precautions would be required in order to free the reagents from traces of tin and lead.

Results of a series of determinations of tin in the presence of varying amounts of lead are shown in Table II. Recoveries are satisfactory even though the concentrations of lead may exceed that of tin by a ratio of 2.5 to 1. The method would obviously not be applicable to the determination of a trace of tin in a relatively gross amount of lead.

RECOVERY DETERMINATIONS

A considerable number of samples representing a wide variety of food products have been analyzed both before and after the addition of measured amounts of tin. Most of these samples originally contained considerable tin; consequently, the results of the recovery determinations in these cases must be regarded as only circumstantial evidence of the precision of the method, since compensating errors might still exist. In a few cases, the

Table III. Results of Recovery Determinations

Product	A Original Tin Content	B Tin Added	C[a] Tin Present	D Tin Found	E Recovery[b]
	P.p.m.	*P.p.m.*	*P.p.m.*	*P.p.m.*	%
Evaporated milk	25.8	25.0	50.8	52.3	103
	27.0	25.0	52.0	53.0	102
	39.1	25.0	64.1	63.9	100
	41.5	50.0	91.5	90.8	99
	46.7	25.0	71.7	72.4	101
	58.4	25.0	83.4	83.1	100
	62.3	25.0	87.3	88.0	101
	69.3	25.0	94.3	92.2	98
	82.6	25.0	107.6	107.6	100
Whole milk	0.4	5.0	5.4	5.4	100
Grapefruit juice	83.4	25.0	108.4	108.0	100
	98.0	25.0	123.0	122.0	99
Prunes	23.8	50.0	73.8	72.0	98
	21.2	15.0	36.2	36.0	101
	24.2	15.0	39.2	39.0	99
	2.1	10.0	12.1	12.0	99
Apricot preserves	1.4	10.0	11.4	11.4	100
Beans with pork	8.9	10.0	18.9	18.7	99
Beets	2.1	10.0	12.1	12.0	99
Mixed vegetable juices	15.6	15.0	30.6	31.8	104
	6.2	25.0	31.2	31.0	99
	3.1	25.0	28.1	27.8	99
Mixed vegetables	0.8	10.0	10.8	10.8	100
Black eyed peas	8.7	10.0	18.7	18.0	96
	6.0	10.0	16.0	16.5	103
Green peas	0.0	25.0	25.0	24.2	97
	0.0	25.0	25.0	23.9	96

[a] Values in C are equal to the sum of those in A and B.
[b] Recovery percentages are obtained from the ratio of figures shown in D to corresponding values in C.

initial tin content of the product was negligible and the fact that results of recovery determinations on these samples were uniformly acceptable more clearly indicates the precision which may be expected. Satisfactory recoveries have been obtained on samples containing as much as 400 parts per million of tin.

The data presented in Table III show the tin content in the original sample, the amount of tin added, and the amount found, all expressed in terms of parts per million. The ratio of the amount actually found to the sum of the original tin content and the added tin is given as the recovery.

DISCUSSION

By means of the procedure described it is possible to determine the tin content of a 10-gram sample of food product containing as little as 0.5 part per million. This represents a considerable increase in sensitivity over the volumetric iodometric method, which requires 50 to 100 grams of material. The increased sensitivity of the polarographic procedure in permitting the use of small samples makes possible a considerable saving in time and a marked economy of reagents. The reduction in sample weight, for example, reduces the quantity of nitric and sulfuric acids consumed in the digestion to less than one fifth that required in the volumetric method. The use of hydrogen sulfide with the accompanying problem of fume removal is eliminated, as well as the time-consuming filtration of the sulfide precipitate. The reduction in sample size also permits the use of smaller vessels and enables the operator to handle three to four times as many determinations concurrently.

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RAW SUGAR

Sampling, Mixing, and Testing

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New practices relating to the sampling, mixing, and testing of undamaged raw sugar imported in bags became effective in the Bureau of Customs in 1943. At the same time a new procedure for controlling the mixing and testing was introduced. The new practices include reduction in the sampling ratio from 100% to one bag in seven and the optional use of a new method of mixing. Experimental work on which the new practices are based is described. Data on the efficacy of the mixing and testing, as revealed by the new control procedure, are given.

THE customs duty on imported raw sugar is based on the direct polarization. In the case of raw sugar in bags, the samples for analysis are obtained from mixtures of portions taken by means of a trier (*6*) inserted into the bags. The Customs Regulations of 1907, 1923, 1931, and 1937 provided that 100% of the bags be sampled.

In 1942, as the result of requests of several government agencies that all possible steps be taken to conserve jute bags because of war conditions, W. R. Johnson, Commissioner of Customs, initiated a systematic study of the sampling, mixing, and testing of raw sugar to ascertain if the percentage of bags sampled per cargo could be reduced without jeopardy to the revenue and the number of bags damaged by customs samplers thereby lessened. The study was developed and conducted by the bureau's Division of Laboratories under the direction of H. J. Wollner (then consulting chemist to the Secretary of the Treasury). The evaluation of the results was made by the application of statistical principles employing standard methods (*1*).

SAMPLING

Precision Using Customs Trier. The preliminary study involved the determination of the degree of variability of the sugar, from bag to bag, in two different cargoes, A and B. Similar information was subsequently obtained on a third cargo, C. The study was designed to reveal information regarding the number of bags which would have to be sampled to ensure that the maximum gain or loss in revenue per cargo due to sampling errors, with a given degree of probability, would not be excessive.

Cargo A appeared to contain a considerable amount of sugar which had been stored for some time prior to export; it was not very uniform in appearance. The sugar in cargo B was generally uniform in appearance. The sugar in cargo C appeared to represent an abnormal case, varying from moist dark brown to dry almost-white sugar.

The three cargoes were sampled and tested by customs officers at Philadelphia, the samples being taken with the customs short trier (*6*), and the tests being made by the customs laboratory method (*8*). One core was taken from each bag sampled and placed in a 2-ounce glass container, which was then sealed with Scotch tape. The cores were taken at regular intervals from

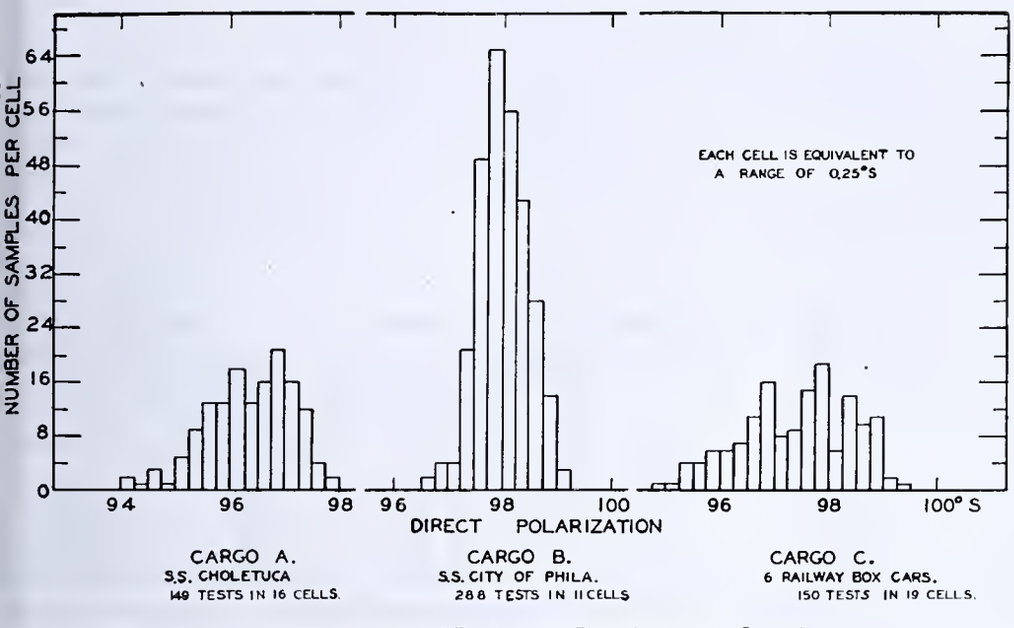

Figure 1. Histograms of Frequency Distribution in Sampling

Table I. Summary of Sampling Data for Three Different Cargoes Using Customs Short Trier

Cargo	No. of Bags	No. of Cores[a]	No. of Groups	Average Test, ° S.	$\acute{\sigma}$, ° S.	Difference Between Highest and Lowest Individual Test, ° S. Actual	Statistical[b]
A	8,996	149	17	97.360	0.718	3.82	4.31
B	19,530	288	32	98.025	0.474	2.47	2.84
C	1,800	150	30	97.157	1.059	4.36	6.35

[a] Also number of tests.
[b] Based on $\pm 3\acute{\sigma}$.

Table II. Summary of Sampling Data for Cargo C Using Three Different Types of Triers

Trier	No. of Cores[a]	Average Test, ° S.	$\acute{\sigma}$, ° S.
Customs short form	150	97.157	1.059
Modified customs short form	150	97.136	1.022
Canadian	150	97.141	1.053

[a] Also number of tests.

different parts of the bags, and each sample was thoroughly mixed before testing. The data are summarized in Table I. Histograms, showing frequency distributions, appear in Figure 1.

The universe standard deviations, $\acute{\sigma}$, shown in Table I represent the variation of the sugar in each cargo and are referred to as sampling precisions. A relatively minor part of the indicated variation is incidental to the testing (see Table IX).

Precision Using Different Triers. The data for cargo C were obtained in a study of the relative efficiency of three different types of triers, the customs short trier, a modified customs short trier, and a Canadian sugar trier. The modification of the customs trier consisted of building up one of its sides by welding on a strip of sheet metal, in the hope of obtaining a more representative sample. The Canadian trier was furnished to the bureau by one of the government war agencies interested in the conservation of jute. It had a solid pointed tip, was longer and narrower than the customs trier, and was said to cause less damage to the bags during sampling. In this study one trier was assigned to a sampler and three cores were taken from each bag sampled—one core for each trier. In terms of sampling precision, the three triers appeared to be about equal (Table II).

While the Canadian trier caused less damage to the bags, it was found unsuitable for use in sampling hard, gummy sugar, which was being imported in quantity at the time. An endeavor to eliminate this objection was made by cutting off the solid tip. While cores could be obtained from all types of raw sugar, using the modified Canadian trier, dry sugar had a tendency to slip off when the trier was removed from the bag, and some sugars caked in the spoon of the trier and were rather difficult to detach. Accordingly this phase of the sampling study was terminated, and all subsequent tests were made with the customs short trier.

Maximum Precision. The maximum universe standard deviation found in the study of cargoes A, B, and C was 1.059° S. The cargo for which this figure was obtained undoubtedly comprised abnormally nonuniform sugars. In view of this, and since customs experience over a period of years indicates that cargoes of raw sugar in normal times are generally more uniform, 1.1° S. is considered a conservatively high figure for the variation among bags in a cargo of undamaged raw sugar. The only published information on this subject which has come to the attention of the writer is that developed by Vondrak (*11*) and by Browne and Zerban (*2*).

Probable Effects of Reduced Sampling. A universe standard deviation of 1.1° S. was used in calculating the number of bags of undamaged raw sugar which would have to be sampled at random (one core per bag) to ensure that the maximum sampling error, with a given degree of probability, would not exceed certain figures. The calculated values appear in Table III.

Table III indicates that in order to ensure a sampling error of not more than 0.05° S. in 99 cases out of 100, at least 3212 bags should be sampled, one core per bag. For an average cargo of 20,000 bags this would require the sampling of about one bag in seven. If we assume that the cargo consisted of 20,000 bags of 96° S. Cuban sugar, containing 325 pounds per bag, a sampling error of 0.05° S. would be equivalent to about $37 at the present rate of duty (*10*), or about 0.075% of the total duty involved. According to Table III, if the same cargo were sampled 100%, the sampling error would not exceed 0.02° S. in 99 cases out of 100. This would be equivalent to about $15 at the present rate or about 0.03% of the total duty involved.

The possibility of applying a sampling ratio of one bag in seven to all cargoes of undamaged raw sugar was considered. In this connection the maximum gain or loss in revenue for different sized cargoes of 96° S. Cuban raw sugar due to sampling errors, assuming a sampling ratio of one bag in seven and a probability of 0.99, was calculated (Table IV).

Actual Effects of Reduced Sampling. The customs duties which would result from 100% sampling and reduced sampling of Cuban raw sugar in bags were determined from tests made of actual samples from five different cargoes. The sampling, mix-

Table III. Relation between Maximum Sampling Error and Number of Bags to Be Sampled, One Core per Bag[a]

Maximum Sampling Error, ° S.	No. of Bags to Be Sampled (Cores)[b]
0.5	32
0.2	201
0.1	803
0.05	3,212
0.02	20,073
0.01	80,293

[a] Based on $\acute{\sigma}$ of 1.1° S. and probability of 0.99.
[b] Calculated by use of formula: $n = \frac{t^2 \times \acute{\sigma}^2}{E^2}$

where n is number of bags to be sampled (cores), t is factor for probability, $\acute{\sigma}$ is universe standard deviation, and E is maximum sampling error.

ing, and testing were done by customs officers at Philadelphia. In the case of 100% sampling, the usual customs practices (*3*, *8*) were followed, except that the average test of each cargo was calculated on a weight basis rather than on the usual basis of the number of bags. If the mixing practices in effect at the time were applied to the sugar obtained in the reduced sampling experiments, fewer mixes would result and the amount of duty represented by each mix consequently would be greater. This indicated the importance of ensuring thorough mixing in connection with reduced sampling practices—more so than in the case of 100% sampling. With this in mind, the following modifications in the existing practices (*3*, *8*) were introduced in the case of the reduced sampling experiments: each bucket was mixed separately; one laboratory sample was prepared from each bucket; each laboratory sample was obtained by taking portions from different parts of the mixed sugar, using a measuring spoon; the quantity of sugar in each sample was in proportion to the quantity of sugar in each bucket; and the laboratory mixed together the samples from the half-day's sampling of the cargo and, with one exception, made ten tests on the resulting mixture. The results obtained for the five cargoes appear in Table V.

Sampling Ratio Recommended. It was decided to recommend that for customs purposes the sampling of one bag in seven, using the customs short trier, be authorized in the case of undamaged raw sugar. This recommendation was based primarily on the data derived from the statistical study (Table IV) and on actual cargoes (Table V). Data were subsequently obtained on a sixth cargo, sampling 100% and one bag in seven, but were not included in Table V.

MIXING

The over-all error in the average test for a cargo of raw sugar includes, in addition to the sampling error, errors incidental to the mixing and testing operations. Effects of sampling errors have previously been discussed. With regard to mixing and testing errors, for practical reasons these are considered as a combined error in the following discussion.

Precision of Hand Mixing Method. The first step in the study of mixing consisted of determining the precision of the hand method of mixing (*5*) which was in effect at the time. Five cargoes of Cuban raw sugar were involved, one each at Baltimore, New Orleans, New York, Philadelphia, and Savannah. The sampling, mixing, and testing were done by customs officers in the usual manner, except that five tests instead of two were made on each mix of undamaged sugar. The data are summarized in Table VI.

The differences between the highest and lowest average tests of the mixes (Table VI) are an indication of the relative variability of the cargoes. While it might be expected that a definite relationship between the variability of the cargoes and the mixing precisions, $\acute{\sigma}$, would be found, this does not appear to be the case, probably because the testing precision is included in and represents a significant part of the precision of the mixing method.

Table IV. Relation between Maximum Sampling Error and Maximum Gain or Loss in Revenue for Different Sized Cargoes of 96° S. Cuban Raw Sugar (Sampling Ratio 1 in 7)[a]

No. of Bags in Cargo	No. of Bags Sampled (Cores)	Total Revenue, $	Maximum Sampling Error[b], ° S.	Maximum Gain or Loss in Revenue $	Maximum Gain or Loss in Revenue %
50,000	7,143	121,875	0.034	61	0.05
20,000	2,857	48,750	0.053	39	0.08
10,000	1,429	24,375	0.075	27	0.11
5,000	714	12,188	0.106	19	0.16
2,000	286	4,875	0.168	12	0.25
1,000	143	2,438	0.237	9	0.37
500	71	1,219	0.335	6	0.49
300	43	731	0.433	5	0.68

[a] Based on $\acute{\sigma}$ of 1.1° S., probability of 0.99 and 325 pounds of sugar per bag dutiable at $0.0075 per pound.
[b] Calculated by use of formula appearing under Table III.

Table V. Comparison of Revenue for Five Cargoes Based on Actual Tests

Cargo	No. of Bags in Cargo	100% Sampling: No. of tests	100% Sampling: Average test, ° S.[a]	100% Sampling: 1 Revenue	Reduced Sampling: Sampling ratio	Reduced Sampling: No. of tests	Reduced Sampling: Average test, ° S.[b]	Reduced Sampling: 2 Revenue	Difference in Revenue, 1 − 2: $	Difference in Revenue, 1 − 2: %
1	7043	24	96.92028	$ 17,578	3 in 7	40	96.913	$ 17,576	+ 2	0.01
2	15709	48	97.31611	39,397	2 in 7	63	97.342	39,412	−15	0.04
3	24993	74	97.10752	62,557	1 in 7	80	97.106	62,555	+ 2	0.00
4	7124	24	97.01166	17,864	3 in 7	50	96.999	17,861	+ 3	0.02
5	49483	138	97.18038	124,038	1 in 7	80	97.218	124,106	−68	0.05

[a] Calculated to 5 decimals in accordance with practice in effect at the time.
[b] Calculated to 3 decimals in accordance with present practice.

Table VI. Comparison of Mixing and Testing Precisions, $\acute{\sigma}$, for Different Ports (Hand Method of Mixing)

Port Symbol	No. of Mixes	Average Test of Cargo, ° S.	Difference between Highest and Lowest Average Tests of Mixes, ° S.	$\acute{\sigma}$, ° S.
A	36	97.528	0.484	0.0409
B	33	97.324	0.828	0.0658
C	20	97.549	0.142	0.0536
D	21	97.188	0.484	0.0479
E	15	96.864	0.160	0.0438

Precision of New Mixing Method. The customs regulations in effect at the time (*6*) required that the average test be determined for the sugar represented by each half-day's sampling of a cargo and that not more than 3 full buckets of sugar should be mixed together (*7*). This meant that under 100% sampling a number of mixes were generally necessary for each half-day's sampling of a cargo. It was anticipated that a similar condition might also apply when one bag in seven was sampled. Since by limiting the number of mixes per cargo to one each half-day the over-all work would be simplified, it was decided to develop a method of mixing which would serve this purpose in most cases and yet be sufficiently precise.

While the modifications in existing practices referred to in connection with Table V resulted in one mix per half-day, it was believed practicable to devise a simpler method. Accordingly, a number of mixing experiments were subsequently conducted by customs officers at Philadelphia. These experiments culminated in the development of special equipment and a new method of mixing, which was called the box and screen method (*5*). (The method employs a 0.375-inch mesh wire screen, fitted over a 4-box receiver, each box being detachable. With the aid of a similar receiver all the sugar is screened and quartered three times.) The most important experiment is described in the following:

Two abnormal mixtures, each consisting of about 37.5 pounds of low test raw sugar (95° S.) and about 37.5 pounds of high test raw sugar (97.7° S.) were used. In each case 5 bucketfuls (about 75 pounds) were prepared, each containing approximately equal layers of the low and high test sugar. Each lot was mixed by the box and screen method, with the exception that one lot was screened twice instead of three times. In each case 30 groups of samples, each group consisting of one sample from each of the four boxes, were taken from the final mixture. The four samples in each group were taken at the same time. Each sample consisted of about 2 ounces. All the samples in each mixture were tested as a unit, without mixing, by the same chemist, under the same conditions. In the case of two screenings the elapsed time for the mixing was 3.33 minutes and the mixing precision, $\acute{\sigma}$, was 0.1701. In the accepted mixing procedure involving three screenings 5.67 minutes were consumed and the precision was found to be 0.1102.

The precision, $\acute{\sigma}$, of 0.1102 for the box and screen method of mixing represents an extreme condition. In view of this and the results obtained in the study of the hand method of mixing, 0.11° S. may be considered a conservatively high figure for the

Table VII. Relation between Number of Tests per Mix and Maximum Mixing and Testing Error per Mix[a]

No. of Tests per Mix	Maximum Mixing and Testing Error per Mix, ° S.[b]
2	0.200
4	0.142
6	0.116
8	0.100
16	0.071
32	0.050
64	0.035
128	0.025
256	0.018

[a] Based on $\acute{\sigma}$ of 0.11 and probability of 0.99.
[b] Calculated by use of formula appearing under Table III.

ombined mixing and testing precision, $\acute{\sigma}$, when either method is roperly used on undamaged raw sugar. An idea of the relative fficiency of the two methods can be obtained by reference to Table X.

TESTING

Mixing and Testing Errors. Using 0.11 as the mixing and esting precision, the maximum mixing and testing errors per mix or different numbers of tests per mix, for a probability of 0.99, vere calculated (Table VII).

NEW CUSTOMS METHOD

Sampling and Mixing Procedures. Detailed instructions n new procedures for the sampling and mixing of raw sugar or customs purposes were prepared as an amendment to the Bureau of Customs Sampling Guide. This amendment (*5*), in he form of method 17, entitled "Raw Sugar", became effective uly 14, 1943.

Instructions on Testing. The maximum gain or loss in evenue for a cargo of raw sugar due to the mixing and testing rror depends in part on the number of mixes, the quantity of ugar in each mix, and the number of tests per mix. In view of his, and since the amount of revenue represented by a cargo de- ends partly on the quantity of sugar involved, it was deemed mpractical to require that the number of tests per mix or per argo be such that the maximum mixing and testing error, with a iven degree of probability, would not exceed a specified figure. The instructions (*9*) finally issued in this connection were based n a practical application of statistical principles. The pertinent art of these instructions is as follows:

The sample submitted to the laboratory for a mix may consist f four full cans, two full cans, one full can, or even one partially ull can, depending upon the quantity of sugar present in the mix. Two tests shall be made on each can submitted from each mix of ndamaged raw sugar, except that in the case of mixes represent- ng 5000 or more bags, four tests shall be made on each can.

Inasmuch as it is desired to obtain data on the efficacy of the revious mixing of the sample, special care shall be taken to avoid ixing the sugar in the can either before or when removing a por- ion for test. All reasonable efforts shall be made to take each est portion from a different position in the can. For example, fter the upper surface layer has been discarded, the first test ortion shall be taken from the resulting exposed surface. The ucceeding surface layer shall then be discarded before the next ortion is removed for weighing.

The requirement as to the number of tests per can was de- gned to keep the errors incidental to the mixing and testing as ow as practicable. For this purpose it was desirable that each est portion come from a different part of the mix, preferably by aving the number of cans sent to the laboratory for each mix orrespond to the number of tests to be made on each mix. It as deemed impractical at the time to submit one can per test ecause of the difficulty in getting proper containers. In order o accomplish the desired purpose and at the same time permit a tudy of the efficiency of the routine mixing and testing opera- tions, instructions regarding the nonmixing of the samples in the laboratory were included.

Estimated Over-all Precision. It was anticipated that, following the practical application of method 17, the mixing and testing error for an average cargo generally would not exceed the maximum sampling error for the cargo. For example, according to Table IV, the sampling error for a 20,000-bag cargo, sampling ratio one bag in seven, would not be expected to exceed 0.053° S. in 99 cases out of 100. Assuming five as the average number of mixes, each representing 4000 bags, and eight tests per mix, the mixing and testing error for the cargo would not be expected to exceed 0.045° S. in 99 cases out of 100. This figure is obtained by dividing the maximum mixing and testing error per mix (from Table VII) by the square root of the number of mixes. It may also be assumed that the sampling, mixing, and testing error for the cargo would not exceed 0.069° S. in 99 cases out of 100. This figure is obtained by taking the square root of the sum of the squares of the maximum sampling error and the maximum mixing and testing error.

In view of the foregoing, it would appear reasonable to assume that the maximum sampling, mixing, and testing errors due to chance, when method 17 is followed, generally will not be excessive.

CONTROL OF MIXING AND TESTING

Control Procedure. Under the reduced sampling procedure authorized by method 17 each mix represents a greater proportion of the cargo and the total number of mixes required for the cargo are materially less than when 100% sampling is practiced. This indicated the desirability of maintaining some kind of statistical control in connection therewith. Accordingly a procedure designed to reveal inadequacies in the routine mixing and testing of each importation of undamaged raw sugar was devised (*4*), and made official for customs purposes on July 14, 1943. The following are the pertinent portions of the procedure:

When imported raw sugar is sampled for test at each port, the appraiser forwards to the director of the National Bureau of Standards a duplicate of one of the samples sent to the customs laboratory.

Only one sample is submitted to the National Bureau of Standards for each importation. It is taken from one of the regular mixes of undamaged sugar and consists of the same number of cans as the regular laboratory sample from that mix.

The samples sent to the customs laboratory and the National Bureau of Standards are tested immediately on receipt. The average tests and the individual tests obtained on the duplicate samples are forwarded to the U. S. Customs Laboratory, Baltimore, Md., where they are studied by statistical methods.

The customs laboratories do not know which mixes are being studied until after their reports have been submitted to the appraisers. Accordingly, their results represent routine conditions for the testing.

Data, Criteria, and Conclusions. The more important of the statistical values derived from the control procedure will be found in Tables VIII, IX, and X.

Some of the criteria which were set up in connection with the study of the data and the conclusions which were drawn from the study are as follows:

If the standard deviation, σ, for both the customs and the National Bureau of Standards tests for a given sample are out of control, existence of disturbing factors outside the laboratory, presumably in the mixing operation, is indicated. Only three such cases were noted among the 399 samples which could be considered.

If the difference between the average tests (customs and N. B. S.) on the same sample exceeds the range of the control limits for averages, $2A\acute{\sigma}$, an assignable cause in the nature of a constant testing error or a change in the moisture content is indicated. In determining the range of the control limits for averages the higher of the two $\acute{\sigma}$'s was used. Assignable causes were indicated in 28% of the cases when the hand method of mixing was involved, and

Table VIII. Comparison of Average Tests for Individual Customs Chemists and Individual Customs Laboratories with Those for N.B.S.[a]

Customs Laboratory Symbol	Customs Chemist Symbol	No. of Samples	Average Test, ° S. 1 Customs	2 N.B.S.	Difference 1 − 2
A		57	97.158	97.185	−0.027
	H	8	97.404	97.345	+0.059
	I	49	97.118	97.159	−0.041
F		99	97.401	97.343	+0.058
	J	86	97.403	97.334	+0.069
	K	2	97.000	96.936	+0.064
	L	11	97.460	97.487	−0.027
B		128[b]	97.138	97.139	−0.001
	M	6	96.754	96.738	+0.016
	N	46	97.296	97.284	+0.012
	O	76	97.073	97.082	−0.009
C		120	97.218	97.149	+0.069
	P	14	97.058	96.941	+0.117
	Q	106	97.239	97.177	+0.062
D		150	97.369	97.356	+0.013
	R	144	97.370	97.358	+0.012
	S	6	97.353	97.298	+0.055
G		7	98.576	98.710	−0.134
	T	6	98.556	98.705	−0.149
	U	1	98.696	98.738	−0.042
E		64	96.997	96.940	+0.057
	V	21	96.934	96.857	+0.077
	W	24	96.962	96.904	+0.058
	X	19	97.112	97.078	+0.034
Total		625			
Averages			97.254	97.227	+0.027

[a] Table is based on data for samples obtained by both methods of mixing. Number of tests per sample varies from 2 to 8.
[b] Includes samples from 2 ports.

in 34% of the cases when the box and screen method was involved. For these, 70% of the differences were 0.2° S. or less, 95% were 0.3° S. or less, 98% were 0.4° S. or less, 99% were 0.5° S. or less, and none exceeded 0.583° S.

The following conclusions can be drawn from Table X: There is little difference in the precision of the mixing by customs officers at the several ports; and in terms of mixing precision there is little difference between the two methods of mixing as practiced by customs officers.

The $\acute{\sigma}$'s in Tables IX and X can be used in calculating the maximum mixing and testing error per mix for a given probability. However, since the $\acute{\sigma}$'s are based on two tests per can per sample, there may be some question as to the reliability of the error thus determined. Accordingly, a number of the $\acute{\sigma}$'s in Table IX were recalculated, using the data for the first test on each can per sample. Table XI indicates that, as anticipated, the effect of making two tests per can under the prescribed conditions is equivalent to that of making one test on each of two cans.

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Table IX. Comparison of Mixing and Testing Precisions, $\acute{\sigma}$, for Each Method of Mixing Based on Tests by N.B.S. and Customs[a]

Laboratory Symbol	Customs Chemist Symbol	Hand Method No. of samples	$\acute{\sigma}$	Box and Screen Method No. of samples	$\acute{\sigma}$
A	I	32	0.064	..	...
N.B.S.		32	0.039	..	...
F	J	76	0.060	..	...
N.B.S.		76	0.040	..	...
F	L	30	0.064	..	...
B	O	..	...	41	0.061
N.B.S.		..	...	41	0.050
B	O	..	...	58[b]	0.060
N.B.S.		..	...	58[b]	0.049
B	N	..	...	34[b]	0.044
N.B.S.		..	...	34[b]	0.042
C	Q	..	...	106	0.065
N.B.S.		..	...	106	0.046
C	P	..	...	37	0.088
D	R	41	0.047	103	0.044
N.B.S.		41	0.043	103	0.042
D	S	..	...	30	0.055
G	T	34	0.041	..	...
E	V	..	...	34	0.041
	W	..	...	47	0.051
	X	..	...	49	0.066

[a] Same samples used in each case where N.B.S. data are given. In such cases, each sample came from a different cargo. Where no N.B.S. data are given, all samples were not tested by N.B.S. and more than one sample may have come from the same cargo.
[b] Includes samples from 2 ports.

Table X. Comparison of Mixing and Testing Precisions, $\acute{\sigma}$, for Each Method of Mixing Based on N.B.S. Tests

Port	Hand Method No. of samples	N.B.S., $\acute{\sigma}$	Box and Screen Method No. of samples	N.B.S., $\acute{\sigma}$
A	38	0.038	...	...
F	87	0.040	...	...
Y	...	...	33	0.045
B	...	...	63	0.049
C	...	...	120	0.046
D	43	0.043	107	0.041
E	...	...	63	0.047
All	202	0.040	423	0.044

Table XI. Comparison of Mixing and Testing Precisions, $\acute{\sigma}$, for Each Method of Mixing Based on Customs Tests

(2 tests per can and one test per can per sample)

Laboratory Symbol	Customs Chemist Symbol	No. of Tests per Can	Hand Method No. of samples	$\acute{\sigma}$	Box and Screen Method No. of samples	$\acute{\sigma}$
A	I	2	32	0.064	..	...
A	I	1	32	0.056	..	...
F	J	2	76	0.060	..	...
F	J	1	76	0.063	..	...
B	O	2	..	...	41	0.061
B	O	1	..	...	41	0.063
B	O	2	..	...	58[a]	0.060
B	O	1	..	...	58[a]	0.062
B	N	2	..	...	34[a]	0.044
B	N	1	..	...	34[a]	0.045
C	Q	2	..	...	106	0.065
C	Q	1	..	...	106	0.069
D	R	2	41	0.047	103	0.044
D	R	1	41	0.055	103	0.049

[a] Includes samples from 2 ports.

Picric Acid Method for Determination of Aromatic Content of Aviation Gasoline

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Dobryanskii and Tikhonov-Dubrovskii utilized the difference in solubility of picric acid in aromatic and nonaromatic hydrocarbons to develop a method for determining the aromatic content of aviation gasoline. The method has been modified to increase its accuracy, precision, and rapidity. The solubility of the picric acid in gasoline is determined at some temperature between 20° and 30° C. Applying a temperature correction to obtain the solubility at 25° C., the percentage of aromatics is obtained by reference to a standard curve. The presence of olefins and naphthenes necessitates a slight correction: −1.4% for each 10% of olefin and −0.6% for each 10% of naphthenes. The method is accurate to ±1% in the range of 0 to 25% of aromatics.

A METHOD for determining the aromatic content of aviation gasoline by utilizing the difference in solubility of picric acid in aromatic and nonaromatic hydrocarbons was reported by Dobryanskiĭ and Tikhonov-Dubrovskiĭ (*2*). Their method for determining the dissolved picric acid was tedious, and they did not consider the effect of variations in temperature on the solubility. The authors have developed a more accurate, more precise, and more rapid method for determining the dissolved picric acid. By making a temperature correction, the solubility of the acid may be determined at any convenient temperature between 20° and 30° C. (a wider range may be used if needed). The per cent of aromatics in the gasoline is then obtained directly from a curve of aromatic content versus solubility.

The procedure is most accurate in the determination of a single aromatic hydrocarbon in paraffin hydrocarbons, but is satisfactory for those mixtures of aromatics which may be found in aviation gasoline. The presence of olefins and naphthenes in gasoline necessitates a slight correction: −1.4% for each 10% of olefins and −0.6% for each 10% of naphthenes. For high accuracy, therefore, the bromine number of cracked gasolines should be determined and the appropriate correction made.

METHOD

A 60-ml. sample of the gasoline is measured into a 125-ml. Squibb-type separatory funnel containing 1 gram of pure picric acid. Commercially available C.P. or reagent quality picric acid containing up to 10% water gives precise results, but for a large number of determinations the dry picric acid is more convenient. The sample is brought to room temperature, which should be between 20° and 30° C., and is shaken for 5 minutes, either by hand or by means of a mechanical shaker. If shaken by hand, the flask is held in the finger tips, so that the heat from the hand will not cause a change in temperature during the shaking period. The temperature of the sample is then recorded and the sample is filtered into a 125-ml. Erlenmeyer flask through a Whatman No. 1 or other fast filter paper. A 50-ml. sample of the filtrate is pipetted into a 250-ml. iodine flask containing 40 ml. of distilled water. Three drops of *m*-cresol purple indicator are added, and 0.05 *N* alkali is added to the sample from a buret, while the flask is swirled gently, until the color of the indicator is changed to purple and approximately 1-ml. excess of alkali has been added. The mixture is then shaken vigorously for one minute. The two layers are allowed to separate, after which the excess alkali is titrated with 0.01 *N* hydrochloric acid to the complete disappearance of the reddish-purple color of the indicator as viewed under a tungsten filament lamp.

The dissolved picric acid in 100 ml. of a saturated solution of picric acid in the gasoline is expressed by the equation:

$$\frac{\text{Mg. of picric acid}}{\text{100 ml.}} = 458.2 \times (\text{ml. of NaOH} \times N \text{ NaOH} - \text{ml. of HCl} \times N \text{ HCl})$$

The measured solubility is corrected to the value for 25° C. by the equation:

$$\text{Solubility at } 25^\circ \text{ C.} = \frac{\text{mg. of picric acid}}{\text{100 ml.}}[1 + 0.035(25^\circ \text{ C.} - t)]$$

where t is the observed temperature in degrees Centigrade. The percentage of aromatics in the gasoline is then read from one of the curves presented with this report, as discussed below.

EXPERIMENTAL

The development of the method included the following investigation:

Titration of the picric acid dissolved in the gasoline.
Effect of temperature on the solubility of picric acid.
Satisfactory shaking time to saturate gasoline with picric acid.
Effect of purity of picric acid on its solubility in gasoline.
Solubility of picric acid in blends of aromatics with iso-octane (2,2,4-trimethylpentane, Rohm and Haas).
Effect of olefins and naphthenes upon the solubility of picric acid.

SELECTION OF TITRATION METHOD. In this portion of the work the following points were studied:

The most suitable indicator for the titration.
Direct titration of the picric acid compared with back-titration of an excess alkali.
Size of the gasoline sample.
Concentration of alkali.

A blend of 10% benzene in iso-octane was prepared and 500 ml. were shaken with 5 grams of picric acid for 30 minutes. Samples of 10 to 15 ml. of this gasoline, saturated with picric acid, were pipetted into 125-ml. iodine flasks containing water and the acid was titrated: (1) by adding 0.01 or 0.05 *N* alkali with long shaking until an end point was reached with phenolphthalein indicator, or (2) by adding an excess of alkali as evidenced by the color change of thymol blue, bromothymol blue, phenolphthalein, or *m*-cresol purple, and back-titrating with 0.01 *N* acid.

These tests showed that thymol blue did not give a satisfactory end point, probably because it was soluble in the gasoline; that bromothymol blue had a green to yellow end point that was very difficult to detect in the picric acid solution; and that a higher solubility was obtained by using phenolphthalein than by using *m*-cresol purple. This difference may be attributed to differences in the pH range of the two indicators, to the fact that more carbon dioxide may be titrated as picric acid by using phenolphthalein, and that the *m*-cresol purple end point was more easily detected than the phenolphthalein end point. The selection of *m*-cresol purple made it necessary to use the back-titration procedure, since it was impossible to extract all the picric acid from the gasoline phase at the alkaline pH range of this indicator (pH 7.4 to 9.0). The back-titration procedure had a further advantage in that the solution required less shaking to reach the end point.

In varying the size of the gasoline sample for titration from 10 to 50 ml., it was noted that the larger the sample the lower the apparent solubility of picric acid. This effect was attributed to interference by small amounts of carbon dioxide dissolved in the water. Although it would be possible to correct for this aliquot error empirically, it seemed advisable to use the larger gasoline sample.

In comparing results obtained by using 0.01 *N* and 0.05 *N* alkali, it was found that the more concentrated alkali gave re-

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sults of higher precision. It is probable that the 0.01 N alkali contained sufficient carbonate to cause the poorer results. Therefore, 0.05 N alkali is recommended.

DETERMINATION OF TEMPERATURE COEFFICIENT. Measurements of the solubility of picric acid were made at room temperature and at approximately 0° C., in blends of aromatics with technical grade iso-octane and in Houdry process gasoline. These solubilities were inserted into the ideal solubility equation to obtain a constant by which solubilities determined near 25° C. may be corrected to give the solubility at 25° C.

In using ideal solubility Equation 1, weights W_1 and W_2 in milligrams of picric acid per 100 ml. of gasoline have been substituted for mole per cents n_1 and n_2, since

$$\log \frac{n_1}{n_2} = \frac{\Delta H(T_1 - T_2)}{2.303R(T_1T_2)} \quad (1)$$

the solubilities are low. The constant, $\Delta H/2.303R$, has been designated by S, giving the equation the following form:

$$\log \frac{W_1}{W_2} = S\,\frac{(T_1 - T_2)}{(T_1 - T_2)} \quad (2)$$

After solving for S in Equation 2, using the experimental values from Table I, the fractional increase in solubility per degree at 25° C., $\frac{w}{W}$, has been calculated from Equation 3,

$$\log\left(1 + \frac{w}{W}\right) = \frac{S}{298^2} \quad (3)$$

Since the variation of solubility with temperature over a restricted range will be practically linear, solubilities determined between 20° and 30° C. may be corrected to 25° C. by the relation:

$$\text{Solubility at 25° C.} = \text{observed solubility}\left[1 + \frac{w}{W}(25 - t)\right] \quad (4)$$

where t is the observed temperature in degrees Centigrade.

Table I gives the data obtained and the calculated values of w/W.

A value of 0.035 for w/W is recommended for correcting solubilities to 25° C., but should not be used for solubilities measured outside the range of 20° to 30° C. For work of high accuracy it is recommended that the temperature be maintained at 25° C. by means of a constant-temperature bath.

DETERMINATION OF SHAKING TIME. A study was made to determine the minimum shaking time in which a solution of picric acid in gasoline would reach equilibrium. It was found that for a fuel high in aromatics, such as Houdry process fuel, the amount of picric acid dissolved in the gasoline was almost at a maximum after 5 minutes of shaking, being only 1 to 2% greater after 20 minutes. For the iso-octane (0% aromatics), however, a 2-minute shaking period was as adequate as 5 minutes; only a slight increase in solubility was noted after a longer shaking period. A 5-minute period has been selected as the most satisfactory.

Table I. Temperature Coefficient Data

Gasoline	Temperature, ° C., T_1	Solubility, Mg., W_1	Temperature, ° C., T_2	Solubility, Mg., W_2	Temperature Coefficient, Equation 3, $\left(1 + \frac{w}{W}\right)$
Iso-octane	23.6	10.9	1.4	4.7	1.035
Benzene and iso-octane blend					
10% benzene	26.6	59.1	0.5	22.0	1.036
25% benzene	26.0	262.3	0.7	119.3	1.029
Xylene and iso-octane blend					
10% xylene	26.6	74.2	0.5	29.2	1.034
25% xylene	26.2	334.6	0.5	167.4	1.025
Houdry gasoline	25.5	167.1	0.5	43.8	1.051

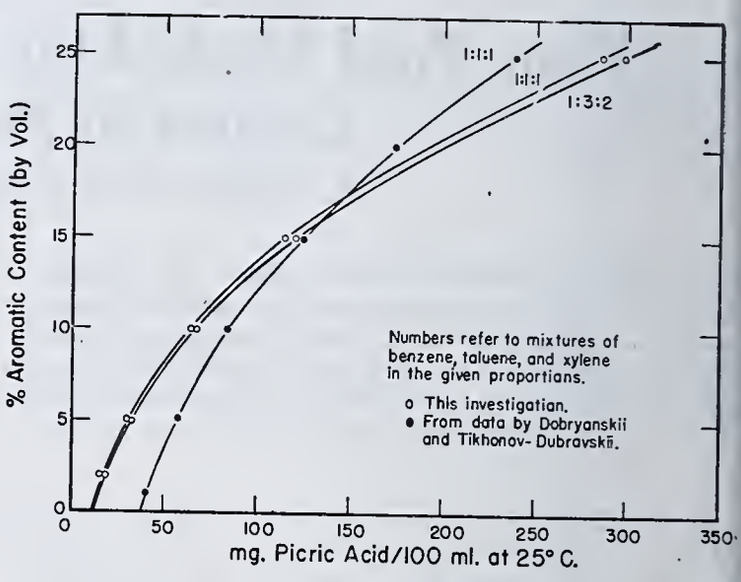

Figure 1. Solubility of Picric Acid

EFFECT OF PURITY OF PICRIC ACID ON ITS SOLUBILITY. Various stocks of picric acid were found to differ in solubility in the same gasoline. Recrystallization increased the solubility of impure samples, but did not change that of the reagent grade material. The solubility of the picric acid is thus a function of its purity.

As shown in Table II, drying the acid has no significant effect on the determination.

SOLUBILITY OF PICRIC ACID IN BLENDS OF AROMATICS. Blends of aromatics with iso-octane were prepared and the solubility of picric acid in these blends was determined by the method described above. The results of these determinations are given in Table III, and are plotted in Figures 1 and 2. All compositions given in this paper are reported on a volume per cent basis.

Table II. Effect of Drying Picric Acid on Its Solubility in Hydrocarbon Blends

Blend	Solubility of Picric Acid, Undried	Solubility of Picric Acid, Dried
	Mg./100 ml.	
A	63.2	63.5
	61.4	62.1
B	297.1	299.2
	301.4	298.9
	301.5	298.2

Table III. Determination of Aromatics in Blends

Aromatic Blends with Iso-octane, %		Solubility of Picric Acid, 25° C., *Mg./100 ml.*
Aromatics	0	11.0
Benzene	5	26.9
	10	55.8
	20	163.0
	25	253.1
Toluene	3	23.1
	10	69.3
	20	198.8
Xylene (commercial)	3	24.0
	10	70.2
	20	206.5
	25	320.5
1:1:1 mix[a]	2	17.3
	5	31.5
	10	64.0
	15	115.4
	25	286.2
1:3:2 mix[a]	2	18.5
	5	32.8
	10	67.3
	15	119.8
	25	297.8

[a] A mixture of benzene, toluene, and commercial xylene in given proportions.

Table IV. Solubility of Picric Acid in 10% Blends of Aromatics in Iso-octane

Aromatic Compound	Solubility of Picric Acid *Mg./100 ml.*	Aromatics (1:3:2 Curve) %	B.P. (Theoretical) ° *C.*
Possible aviation gasoline constituents			
Benzene	56	8.7	80.1
Toluene	69	10.4	110.8
Xylene (commercial)	70	10.5	...
o-Xylene	82	11.8	144
m-Xylene	81	11.7	138.8
m,p-Xylene (mixture)	78	11.3	...
Ethylbenzene	48	7.6	136.2
Cumene	36	5.7	153
Improbable aviation gasoline constituents			
Mesitylene	99	13.4	164.8
Diethylbenzene	41	6.5	182
Triethylbenzene	41	6.5	218
m-Diisopropylbenzene	22	3.0	204
p-Cymene	29	4.4	176
n-Butylbenzene	33	5.1	180
sec-Butylbenzene	26	3.9	174
tert-Butylbenzene	27	4.1	169
sec-Amylbenzene	20	2.6	189
tert-Amylbenzene	24	3.5	190

A plot of the values obtained by Dobryanskiĭ and Tikhonov-Dubrovskiĭ is included in Figure 2 for the sake of comparison. It is believed that the differences in intercept and in slope between their curve and the authors' are due to (1) the presence of olefins or naphthenes in their base stock, (2) the presence in their xylene of some impurity, such as ethylbenzene, in which picric acid is less soluble, and (3) the fact that their determinations were made at temperatures some 10° lower than the authors'.

Besides the three aromatics for which solubility values are reported in Table III, a number of other aromatics were tested in 10% blends with iso-octane. These results are given in Table IV.

Effect of Olefins and Naphthenes. Blends of iso-octane were prepared with cyclohexane and its methyl and *o*-dimethyl derivatives and with amylene, diamylene, diisobutylene, and cyclohexane. Various aromatics were added to these blends, and the solubility of picric acid in the mixture was determined. The apparent content of the aromatic compound or mixture used was then calculated from the curve given by that aromatic in mixture with only iso-octane. The results of these tests, given in Table V, show that both naphthenes and olefins cause a small increase in the apparent aromatic content, the effect of the olefins being greater. The error in terms of per cent aromatics is approximately +1.4% for each 10% of olefins and not more than +0.6% for each 10% naphthenes.

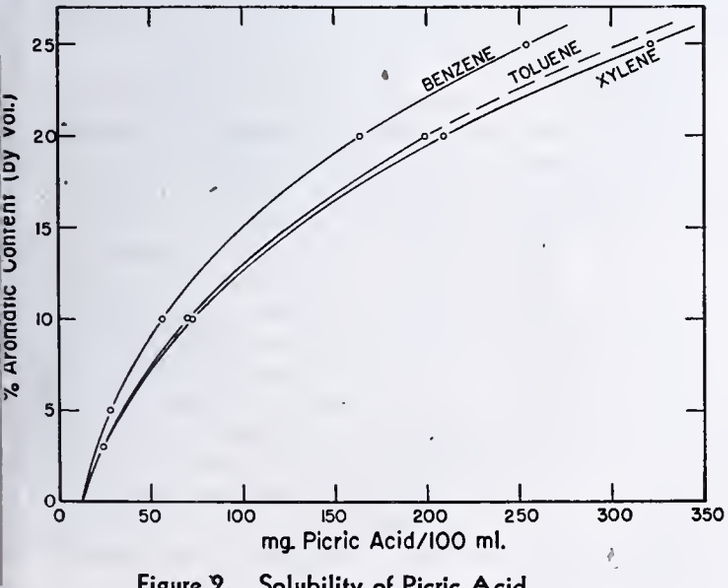

Figure 2. Solubility of Picric Acid

Table V. Effect of Olefins and Naphthenes on Solubility of Picric Acid in Gasoline

	Solubility of Picric Acid *Mg./100 ml.*	Aromatics Calculated from Picric Acid Solubility %
A. Naphtbenes present		
10% benzene, 9% methylcyclobexane, 81% iso-octane	59.1	10.4
10% toluene, 9% methylcyclohexane, 81% iso-octane	73.3	10.5
10% xylene, 9% methylcyclobexane, 81% iso-octane	77.7	10.6
10% 1:1:1[a], 9% metbylcyclohexane, 81% iso-octane	69.7	10.5
10% 1:3:2[a], 9% metbylcyclohexane, 81% iso-octane	71.7	10.5
10%benzene, 45% metbylcyclobexane, 45% iso-octane	85.8	13.8
10% toluene, 45% methylcyclohexane, 45% iso-octane	98.8	13.0
10% xylene, 45% methylcyclobexane, 45% iso-octane	106.4	13.4
10% 1:1:1[a], 45% metbylcyclohexane, 45% iso-octane	94.5	13.2
0% aromatics, 50% methylcyclobexane, 50% iso-octane	23.1	3.0
0% aromatics, 25% cyclobexane	11.3	0
0% aromatics, 25% *o*-dimethylcyclobexane	12.6	0.3
B. Olefins present		
10% 1:1:1[a], 10% amylene, 80% iso-octane	76.0	11.4
10% 1:1:1[a], 25% amylene, 65% iso-octane	100.8	13.7
10% 1:1:1[a], 10% diamylene, 80% iso-octane	77.7	11.5
10% 1:1:1[a], 25% diamylene, 65% iso-octane	97.1	13.4
10% 1:3:2[a], 10% amylene, 80% iso-octane	82.9	11.8
10% 1:3:2[a], 25% amylene, 65% iso-octane	102.3	13.6
10% 1:3:2[a], 10% diamylene, 80% iso-octane	79.3	11.4
10% 1:3:2[a], 25% diamylene, 65% iso-octane	98.7	13.3
0% aromatics, 10% cyclohexene	13.5	0.6
0% aromatics, 25% cyclohexene	22.2	2.9
C. Napbtbenes and olefins present		
10% 1:1:1[a], 25% methylcyclobexane, 10% diamylene, 55% iso-octane	94.8	13.2
10% 1:3:2[a], 25% methylcyclohexane, 10% diamylene, 55% iso-octane	99.0	13.3

[a] A mixture of benzene, toluene, and commercial xylene in given proportions.

Solubility of Picric Acid in Pure Aromatics. The results of measurements of the solubility of picric acid in undiluted aromatics, not especially purified, are given in Table VI.

Analysis of Aviation Gasolines. Since the different aromatic compounds give slightly different solubility curves, it is necessary in applying the picric acid method to gasoline to make some assumption regarding the composition of the aromatics in the gasoline. The authors have assumed that a 1 to 3 to 2 mixture of benzene, toluene, and xylene gives a good average representation of the aromatics in most aviation gasoline, and have

Table VI. Solubility of Picric Acid in Undiluted Aromatics

Aromatic	Solubility of Picric Acid *G./100 ml. aromatic*	Temperature ° *C.*
Benzene	7.550	22.4
Toluene	8.950	22.3
Xylene (commercial)	9.810	22.8

Table VII. Determination of Aromatics in Aviation Gasoline

	Per Cent Aromatics		
Gasoline	Picric acid method	Ultraviolet absorption (*I*)	Sulfonation (*S*)
A	0	0.2	
B	0	< 1	
C	25.0	24	
D	10.6	11	
E	0	< 1	
F	20.6[a]	21.0	21[a]
G	25.2[a]	23.4	24
H	0.8	1.5	2

[a] Corrected for olefin content.

used the curves for this mixture as a standard in determining the aromatic content of a number of typical fuels. Table VII compares results with those obtained on the same gasolines by other workers using ultraviolet absorption and acid absorption methods. Agreement between the different methods is seen to be satisfactory.

DISCUSSION

The chief error in the picric acid method lies not in the reproducibility of the determinations, which is about ±0.2%, but in the difference of solubility of the acid in the various individual aromatic compounds. The solubilities in benzene, toluene, and xylene are sufficiently similar so that no great error is committed by assuming them to be present in the ratio of 1 to 3 to 2, and in the gasolines examined the results appear to be accurate to better than 1%. To obtain more accurate results for the low-boiling constituents, it is only necessary to fractionate the gasoline and determine the benzene and toluene contents separately on the appropriate fractions. However, for the higher-boiling compounds, such as ethylbenzene and cumene, the solubility is low, and in so far as these compounds are present the aromatic content of the gasoline will be underestimated.

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Determination of Olefinic Unsaturation

Nitrogen Tetroxide Method as Applied to Petroleum Hydrocarbons in the Gasoline Boiling Range

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A radically new method has been developed for determining the olefinic unsaturation of petroleum hydrocarbons lying in the gasoline boiling range. It is based on the reaction of nitrogen tetroxide with olefinic materials to convert them into oily addition products, or nitrosates. Separation of the unreacted material, including paraffins, naphthenes, and aromatics, may be effected by steam-distillation or by reaction and solution of the nitrosates in alcoholic potassium hydroxide and potassium sulfide. The change in volume of the sample gives a measure of the olefinic unsaturation.

THE determination of olefinic unsaturation in hydrocarbons has long been a controversial subject in the petroleum industry. Calculations based on the bromine number or iodine number have been accepted generally as the only feasible means of determining such unsaturation. That such methods are not entirely satisfactory is generally realized, as evidenced by the large number of bromine or iodine number procedures which have been proposed; almost every major oil company has its own modification which may give results differing appreciably from those of another company. The nature of the solvent used, temperature, time of contact, excess of halogen present, and many other factors affect the results which are obtained. The nature of the unsaturate itself also is an important factor. In conjugated diolefins, halogenation of both double bonds is usually incomplete and this same uncertainty applies to aromatics with an olefinic side chain. Extensive substitution, rather than simple addition of halogen, also may occur.

The difficulty of translating the bromine or iodine number into per cent olefinic unsaturation likewise is an important factor, due to lack of knowledge, in general, of the average molecular weight of the olefins involved or their distribution throughout the sample. In the case of whole gasoline samples, particularly those which are thermally cracked and may contain a mixture of olefins and diolefins as well as aromatics or even acetylenes, the problem becomes most acute.

A method that will give a direct volumetric measure of the olefinic unsaturation of the hydrocarbon sample, regardless of the type of olefin present, and is without effect on paraffins, naphthenes, or aromatics, would present an almost ideal solution to this problem. Such a method has been developed at this laboratory and has given most promising results on a wide variety of the most difficult and complex types of samples, both synthetic and natural. It also has the additional advantage that it can be used on dark-colored samples.

DESCRIPTION OF METHOD

The author has recently noted that nitrogen tetroxide will react rapidly and completely with all commonly available olefinic materials in the temperature range 104° to 410° F., converting them into heavy, oily addition products, the majority of which are of very low volatility, and from which any unreacted components of the mixture can be removed readily by a simple steam-distillation. The possibility of using nitrogen tetroxide as an analytical tool for resolving complex olefinic-aromatic mixtures at once became apparent and an extended investigation was undertaken.

Two methods finally resulted from this investigation, the distillation method mentioned in the preceding paragraph and a rapid method based on the discovery that the olefin nitrosates will react with alcoholic potassium hydroxide and potassium sulfide to form products soluble in 50% alcohol. It thus becomes possible to measure the volume of the paraffins, naphthenes, and aromatics, which are unaffected by the reagents used in the test, the decrease in volume from that of the original sample giving a measure of the olefin content.

Relatively little information is given in the literature regarding the nature of the products formed by interaction of nitrogen tetroxide and olefins. According to Sidgwick (*4*), such reaction products are known as nitrosates or "Dinitrüre", possessing the structure $\left[\begin{array}{cc}-CH- & -C< \\ | & | \\ NO & O.NO_2\end{array}\right]_2$ or $\begin{array}{cc}>C- & -C< \\ | & | \\ NO_2 & NO_2\end{array}$, respectively. They thus result from direct addition of nitrogen tetroxide to the olefinic double bond. There is some question as to the bimolecular structure of the nitrosates and Riebsomer (*3*) presents conflicting opinions of various research workers. The nature of the solvent appears to play an important role as to which product is formed. However, the exact structure of these compounds is of minor importance for the purpose of this investigation, and they are referred to here merely as "nitrosates". The nitrosates may be hydrolyzed to nitro-alcohols or rearranged to nitro-oximes during steam-distillation.

APPARATUS

A 200- to 250-ml. treating-distillation flask with a spray trap on the outlet and a steam inlet tube, as illustrated in Figure 1. Ground-glass connections are recommended.

Product receiver assembly, consisting of a curved glass adapter (Figure 2) with an extension of 4-mm. glass tubing which will nearly reach the bottom of the 150- to 250-ml. separatory funnel used as a receiver. A short length of 2-mm. capillary glass tubing is sealed in the upper wall of the large end of the adapter as an air inlet. The purpose of this adapter is to cause the drops of distilled oil to issue below the surface of the water in the separatory funnel, thus making evident the point at which these drops of oil become heavier than water. This receiver assembly is not designed for very volatile samples, which may require a more adequate condensing system.

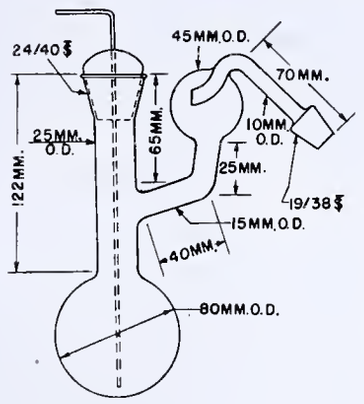

Figure 1. Treating Flask

Reaction Bottle. A modified Babcock bottle (*1*).

REAGENTS

Sulfamic Acid Solution. Dissolve 20 grams of sulfamic acid ($H_2NSO_2.OH$) in water and dilute to 200 ml. (a 25% solution of urea may be used instead, if desired).

Alcoholic Potassium Hydroxide Solution, 10%. Dissolve 20 grams of potassium hydroxide in methanol and dilute with methanol to 200 ml.

Alcoholic Potassium Sulfide Solution. Dissolve 50 grams of potassium hydroxide in about 130 ml. of methanol, cool, and dilute to 160 to 175 ml. Pass hydrogen sulfide into this solution until the weight has increased 15.0 grams (about 18 ml. increase in volume), cool, and dilute to 200 ml. with methanol.

Nitrogen Tetroxide (obtainable in 10-lb. cylinders from Du Pont Co.).

Alkaline Sodium Sulfide Solution. Dissolve 100 grams of sodium hydroxide and 150 grams of sodium sulfide crystals in 800 ml. of water, cool, and add 200 ml. of acetone.

Alcoholic Potassium Hydroxide Solution, 25%. Dissolve 50 grams of potassium hydroxide in methanol and dilute with methanol to 200 ml.

Dilute alcoholic sodium hydroxide solution. Dissolve 40 grams of sodium hydroxide in water, dilute to 100 ml. with water, and add 100 ml. of methanol.

NOTE. Methanol is used in preparing these reagents in preference to ethanol, as solutions prepared with the latter soon turn dark red.

DEVELOPMENT OF THE METHOD

To be of value for determining the olefin content of a gasoline sample, it must be shown that nitrogen tetroxide is without effect on the other components of the sample. Accordingly, a series of known mixtures was prepared, containing various paraffins, naphthenes, olefins, diolefins, acetylenes, aromatics, etc., in the boiling range of gasoline or approximately 104° to 410° F. Most of the materials used were of a commercial grade without any attempt at purification (Table I).

Table I. Test Components

Mixed heptanes	Phillips Petroleum commercial grade (2% soluble in concentrated H_2SO_4)
Cyclopentane	Phillips Petroleum technical
Methylcyclohexane	Dow Chemical Co. technical
Cyclohexene	Dow Chemical Co. technical
Pure *n*-heptane	Westvaco (Bureau of Standards certificate)
2-Methylpentadiene	Commercial Solvents Corp.
Diisobutylene	Eastman Kodak Co. practical
Dicyclopentadiene	Koppers Co.
α-Methylstyrene + cumene	Crude mixture
Dipentene	Hercules Powder Co. (commercial)
p-Cymene	Claflin Co.
Mesitylene	Eastman Kodak Co.
Xylene	General Chemical Co. reagent
Iso-octane	Knock test standard
C_{10} aromatics	Isolated from Houdry gasoline
Phenylacetylene	Eastman Kodak Co.
Phenol	Reagent grade
Benzene	Barrett Co. "pure"
2-Methylpentane	140° F. cut, acid-treated gasoline

This first series of blends was analyzed by a rather crude procedure which subsequently was improved to reduce handling losses. The chilled sample was saturated with gaseous nitrogen tetroxide, washed with water to remove any excess nitrogen tetroxide, transferred to a flask, and steam-distilled into a chilled receiver until the portions of oil coming over were heavier than water. After removal of the water layer, the distilled oil was extracted with 10% sodium hydroxide solution to remove any nitrosate reaction product carried over and the volume of the unreacted hydrocarbon was then measured, applying a correction factor for handling losses. The decrease in volume represented the olefinic unsaturation of the sample. Any aromatics in the treated hydrocarbon could readily be determined by the usual sulfuric acid treating procedure and corrected to the basis of the original sample.

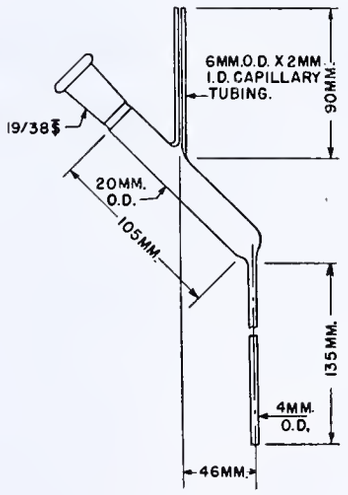

Figure 2. Adapter

In Table II are shown the various blends analyzed by the original nitrogen tetroxide method. As the commercial mixed heptanes used in preparing most of these blends contained 1.8% of material reacting with nitrogen tetroxide, values on such blends were corrected on the basis of the amount of this heptane used. The bromine numbers of most of the blends were determined and calculated as mono-olefin. The "total olefin" value is based on the volume per cent of added olefin, regardless of its actual purity. In the case of dipentene, results indicated the dipentene to have a purity of 88%, and the figure on the α-methylstyrene blend was based on the result of a polymerization test.

Considering the lack of refinements in this original distillation procedure, surprisingly good agreement was shown between the amounts of olefin assumed to be present and those determined, most of the discrepancies probably being due to impurities in the added material. Where diolefins were present, the results obtained were definitely superior to those calculated from the bromine number. It is evident that nitrogen tetroxide is without appreciable action on paraffins, naphthenes, and aromatics boiling in the range 120° to 410° F. As phenol reacts with nitrogen tetroxide, phenolic constituents, if present in appreciable amounts in a sample, should be removed prior to the analysis by a caustic wash. The majority of such phenols as occur in gasoline are soluble in aqueous caustic solution.

PROCEDURE

METHOD A. This method is intended for the volumetric determination of the total olefinic unsaturation of hydrocarbons boiling between 120° and 410° F.

Immerse the clean, dry, treating flask (Figure 1) in an ice bath extending well up around the neck of the flask. Record the temperature of the sample and pipet 50 ml. into the flask. Place the bath and flask under the hood. Place a suitable thermometer covering the range 30° to 120° F. in the sample and introduce nitrogen tetroxide gas well below the surface of the sample through a glass tube drawn out to a long slender 1-mm. capillary, controlling the flow of gas to maintain the temperature of the sample at 80° to 100° F. (Saran or a short length of Tygon tubing has been found satisfactory for connecting the glass delivery tube to copper tubing from the cylinder of nitrogen tetroxide. Rubber should not be used. The cylinder should be maintained at a temperature above 71° F. to facilitate vaporization of the nitrogen tetroxide.) Completion of reaction is indicated by a definite drop in sample temperature to about 70° F., even though the flow of gas is increased, and by marked formation of brown fumes

Table II. Analysis of Known Mixtures by Original Nitrogen Tetroxide Method

Test	Blend	%	Bromine No. of Blend	Calculated Monoolefin Equivalent, %	Total Added Olefin, %	% Olefin Content from Volume Decrease
1	Commercial heptane	100	0.6	.....	0	1.8
2	Heptane Cyclopentane	66.7 33.3	0.3	.....	0	0.6 (corrected for heptane value)
3	Heptane Methylcyclohexane	66.7 33.3	....	.....	0	0.4 (corrected)
4	Heptane Cyclohexene	86.7 13.3	27.2	14.0	13.3	13.2 (corrected)
5	Pure *n*-heptane	100	0	.....	0	0.0
6	Isohexane	100	0	.....	0	1.2
7	Heptane 2-Methylpentadiene	86.7 13.3	29.3	15.4 (equivalent to 1.14 double bonds)	13.3	13.6 (corrected)
8	Heptane Diisobutylene	66.7 33.3	47.4	33.2	33.3	33.4 (corrected)
9	Heptane Dicyclopentadiene	86.7 13.3	33.2	27.4	13.3	13.6 (corrected)
10	Heptane α-Methylstyrene Cumene	66.7 23.7 ±0.3 9.6 ±0.3	38.1	28.1 (equivalent to 1.17 double bonds)	23.7 ±0.3	24.6 (corrected)
11	Heptane Dipentene	66.7 33.3	65.4	49.5 (equivalent to 1.5 double bonds)	33.3 (?)	29.2 (corrected)
12	Heptane *p*-Cymene	66.7 33.3	0.4	.....	0.0	0.2 (corrected)
13	Heptane Mesitylene	86.7 13.3	....	.....	0.0	0.0 (corrected)
14	Heptane Cyclohexene Diisobutylene	50 25 25	92.2	48.2 (94 Mol. wt.)	50	48.7 (corrected)
15	Heptane Cyclohexene 2-Methylpentadiene Xylene	50 20 10 20	60.4	31	30	29.7 (corrected)
16	Heptane C_{10} aromatics (330°–380° F.)	75 25	....	.....	0.0	0.0 (corrected)
17	Iso-octane	100	....	.....	...	1.0
18	Iso-octane Phenylacetylene	96 4	....	.....	4.0	4.6 (uncorrected)
19	Heptane Benzene Phenol	86.7 8.0 5.3	....	.....	...	6.2

above the surface of the sample. (Samples of low olefin content may produce only a slight rise in temperature.) Remove the thermometer and delivery tube, flushing each with small portions of water to prevent loss of sample.

Add sulfamic acid (or urea) solution slowly to the chilled sample, with continuous agitation, until the brown fumes disappear, taking care to avoid loss due to foaming. The sample usually assumes a green or blue color at this point. Connect the flask to an efficient condenser and the receiver assembly and insert the steam inlet tube. Put 10 to 20 ml. of water in the receiver to cover the end of the adapter extension and surround the assembly with an ice bath. Immerse the distillation flask in a beaker of hot water and place a heater under the beaker to bring the water to boiling, then introduce steam into the sample, slowly at first, and adjust the rate as distillation proceeds. The side tube on the adapter should be closed off at the start to prevent loss of vapors and then opened if the water in the receiver starts to suck back. Continue distillation until the distillate becomes heavier than water and sinks to the bottom. (Occasionally a sample is encountered, such as triisobutylene or some polymer gasolines, where the nitrosate distillate is partly lighter than water. In this case, distillation is stopped when the ratio of oil to water in fresh portions of the distillate decreases to about 1 to 4.)

Remove the receiver and carefully draw off any heavy nitrosate and the water. Add 10 ml. of the 10% alcoholic potassium hydroxide to the remaining hydrocarbon, stopper the funnel, and shake vigorously a few moments. Add 25 ml. of water, again shake vigorously, and allow to settle, keeping the funnel cold. Draw off the aqueous layer carefully, add 15 ml. of the alcoholic potassium sulfide to the hydrocarbon, and shake vigorously for 4 minutes. Add 50 ml. of water, shake, and allow to settle as before. Draw off the aqueous layer. Wash the hydrocarbon once more with 50 ml. of water. Drain the hydrocarbon layer into a 50-ml. buret which has been cleaned with chromic-sulfuric acid mixture and well rinsed with water. Rinse the separatory funnel with several small portions of water, thus bringing the hydrocarbon layer well within the graduations of the buret. Immerse the buret for 5 minutes in a suitable water bath (such as a section of 50-mm. glass tubing closed at one end and filled with water) maintained within 2° F. of the temperature at which the test sample was measured. Read the volume of hydrocarbon to the nearest 0.1 ml. and record as volume of unreacted material. If desired, this material may be used, after drying, for determination of density, refractive index, or acid absorption to ascertain the content of aromatics.

As a check on the completeness of olefin removal, it may be advisable to make a duplicate determination. React the residue in the distillation flask with an equal volume of alkaline sodium sulfide solution and discard. To determine the distillation and handling loss, repeat the procedure given above on another 50-ml. portion of the original sample, omitting the treatment with nitrogen tetroxide.

CALCULATION. Calculate the percentage of olefinic unsaturation by means of the following equation:

$$U = \frac{50.0 - (V + C)}{50.0} \times 100$$

where

U = per cent olefins by volume
V = volume of unreacted hydrocarbon, ml.
C = loss correction, ml.

When analyzing samples of polymer gasoline, tri-, or tetraisobutylene, after saturation with nitrogen tetroxide the mixture should be allowed to stand for about 5 minutes before destroying the excess nitrogen tetroxide, since the reaction is slower than in the case of most olefins.

METHOD B. This method is intended for the determination of the total olefinic unsaturation of hydrocarbons boiling between 104° and 410° F. It is directly applicable to samples containing not more than 50% by volume of olefin, and is applicable to samples containing over 50% by volume of olefin, providing they have been diluted with a suitable measured amount of nonolefinic hydrocarbon and the determination is made on the diluted sample.

Immerse the clean dry Babcock reaction bottle in an ice-water bath to about the 60% mark, record the temperature of the sample, and pipet 10.00 ml. into the bottle. Place the bath and bottle under the hood near the nitrogen tetroxide cylinder. Place the thermometer and capillary end of the nitrogen tetroxide delivery tube in the sample and introduce a stream of the gas at such rate that the temperature of the sample is held between 80° and 100° F. Completion of the reaction is indicated by a definite drop in sample temperature to about 70° F., even though the flow of gas is increased, and by the marked appearance of brown fumes above the surface of the sample. (Samples of low olefin content may produce only a slight rise in temperature.) Stop the flow of gas and remove the delivery tube, touching it to the inside of the neck of the bottle to drain off any sample.

Raise the thermometer and flush with 1 ml. of the dilute alcoholic sodium hydroxide solution before withdrawing, to prevent loss of sample.

Table III. Comparison of Analytical Methods on Samples Containing Diolefins

	Usual Method				Nitrogen Tetroxide Method			
	Olefins by bromine no.	Aromatics by specific dispersion	Sum	Acid treat on original	Olefins by volume decrease	Aromatics by acid treat	Sum	Acid treat on original
			Sample 818 AL					
ut 4 (150–205)	52	0	52	46	46	2	48	46
ut 5 (205–250)	46	11	57	49	36	11	47	49
ut 6 (250–302)	40	23	63	57	28	27	55	57
ut 7 (302–347)	28	39	67	61	20	39	59	61
ut 8 (347–396)	22	44	66	61	17	45	62	61
			Sample 806 AL					
ut 5	56	16	72	63	46	18	64	63
ut 6	45	37	82	71	29	43	72	71
ut 7	25	56	81	77	17	51	78	77
ut 8	19	60	79	75	18	59	77	75

Agitate the bottle in the ice bath with a swirling motion and radually add 1 ml. more of the sodium hydroxide solution to ecompose excess nitrogen tetroxide. In a similar manner gradu-lly add 8 ml. of the 25% alcoholic potassium hydroxide solution, ausing the sample to turn dark brown. Moisten the glass stopper f the bottle with a drop of the potassium hydroxide solution, in-ert, and shake the bottle vigorously for 30 seconds. Release ny gas pressure carefully to avoid loss of sample. Gradually add 5 ml. of the alcoholic potassium sulfide with agitation and cool-ng. Stopper the bottle and shake a few moments, release any as pressure, then continue vigorous shaking for 4 minutes. Di-ute the mixture gradually with water (25 to 27 ml.) to bring the nreacted hydrocarbon within the graduations on the neck of the ottle, mix well, release any gas pressure, and centrifuge at 000 r.p.m. for 2 to 3 minutes.

Immerse the bottle for 3 minutes in a water bath maintained ithin 2° F. of the temperature at which the test sample was easured. Read the volume of the hydrocarbon to the nearest % (0.1 ml.) and record as the volume per cent of unreacted sam-le.

CALCULATION. $U = 100 - V$, where U = per cent olefins y volume and V = per cent of unreacted sample.

SAFETY PRECAUTIONS

Vapors of nitrogen tetroxide are toxic (concentrations as low as 00 parts per million may prove fatal); so all operations involv-ng its use should be carried out under an efficient hood.

Nitrogen tetroxide may react with certain organic compounds o form products which are violently explosive. The higher-boil-ng hydrocarbons, including higher aromatics, are much more usceptible to oxidation or nitration than are those in the gasoline oiling range and the nitrosates of some of the terpenes (reaction roducts with turpentine) have been found to decompose spon-aneously. Therefore, prolonged contact of oils or greases with xcess nitrogen tetroxide, which might lead to nitration or perox-le formation, should be avoided. Graphite is recommended as lubricant on any valves or pipe connections which may be in ontact with the gas.

No serious decomposition of the nitrosates of olefins in the gaso-ne boiling range has been observed at temperatures up to 212° F., ith the exception of high concentrations of certain terpenes, lthough many of them show very gradual evolution of fumes on ong standing, even at room temperature. Most severe tests, in-luding the use of detonators, have failed to explode them. They xert a pronounced, although temporary, irritating action if per-itted to come in contact with the mucous membrane or sensitive kin surfaces.

It is felt that petroleum hydrocarbons in the gasoline boiling ange, when handled as specified in this method, present no hazard nd this is substantiated by several hundred analyses in various aboratories. However, the use of a safety shield or at least a face ask is advised.

This method was applied with marked success to a number of aboratory samples evidently containing diolefins, where a marked ifference had been found to exist between the total unsaturation etermined by acid-treating (*1*) and the sum of the apparent ole-fin content calculated from the bromine number and the apparent aromatic content calculated from the specific dispersion (*2*). A comparison of these methods on two such samples appears in Table III.

It is at once evident that far better agreement is found between the sum of aromatics plus olefins determined by the nitrogen tetroxide procedure and the total acid absorption than by the usual method of analysis.

The method has likewise shown its value for the determination of aromatics in polymer gasoline or highly cracked naphthas. Removal of the olefins is readily effected by their conversion to nitrosates and steam-distillation of the unchanged aromatics, paraffins, and naphthenes. The aromatics in the distillate may be then determined in any desired manner or actually isolated, as by preferential adsorption on silica gel or by sulfonation and hydrolysis of the sulfonic acid.

The original method was substantially improved by saturating the sample with nitrogen tetroxide in the distillation flask itself, destroying excess nitrogen tetroxide by the addition of urea or sulfamic acid solution, steam-distilling, and washing the distillate with alcoholic potassium hydroxide and potassium sulfide as described in Method A. Caustic should not be used to destroy the nitrogen tetroxide, since it may react with the nitrosates during steam-distillation to form more volatile oils, which would come over with the unreacted components of the sample and not be removed by the washing step, leading to low results for the olefin content.

This analytical procedure is limited at present to samples lying in the gasoline boiling range: (1) inclusion of lower-boiling fractions would increase and make more uncertain the handling losses, (2) inclusion of material boiling above 410° F. requires the use of excessive amounts of steam for distillation and lessens the sharpness of the split between unreacted hydrocarbon and nitrosate, and (3) certain of the higher-boiling aromatics, such as naphthalene and acenaphthene, may be nitrated or oxidized to an appreciable extent, leading to high results for the olefin content.

Desiring to develop a more rapid procedure which could be used for routine testing without involving the elaborate distillation setup, it was discovered that the oily nitrosates would react with alcoholic potassium sulfide to give compounds which were soluble in 50% alcohol. The reaction could thus be carried out in a Babcock-type bottle, such as is used in determining the acid absorption of gasoline, the nitrosate dissolved by the addition of alcoholic potassium sulfide, and the volume of unreacted sample read in the neck of the bottle after centrifuging. Handling losses were thus reduced to a minimum, only 10 ml. (or 5 ml.) of sample were required, and an average single determination could be made in 20 minutes or less. However, the accuracy of reading was limited to 1% by virtue of the small sample size and design of the bottle, and the maximum olefin content which could be determined was limited by the amount of absorbing reagent which could be used.

Table IV gives a comparison of this rapid procedure with the distillation procedure on several samples.

It was found that blends with triisobutylene and with decene-1 in acid-treated naphtha did not give theoretical results by this rapid procedure; the decene-1 gave rise to an intermediate dark red oil layer and low results were obtained with the triisobutylene. The latter compound also gave trouble in the distillation procedure, because early portions of the distillate of the nitrosate

Table IV. Comparison of Rapid and Distillation Methods

Sample	% Olefins (Rapid Method)	% Olefins (Distillation Method)
Impure heptane-cyclopentane	2	1.8
Heptane-2-methylpentadiene	15	15.2
Heptane-diisobutylene	34	34.2
100° to 150° F. gasoline cut	20	20 (by bromine No.)
Crude benzene	7	8
B-1	25	25.4
B-2	34	34.3
B-3	28	27.5
B-4	28	27.7

were lighter than water, contrary to the behavior of the nitrosate distillate of most olefins.

By shaking this light nitrosate distillate with alcoholic potassium sulfide for 3 minutes, it was rendered soluble in 50% alcohol, this minimum time factor being required for complete reaction. It was also discovered that the nitrosate of decene-1 reacted completely with alcoholic potassium hydroxide to give a compound soluble in 50% alcohol. Therefore, to take care of any type of sample, the rapid method was modified to include treatment with alcoholic potassium hydroxide prior to the addition of alcoholic potassium sulfide and the shaking time with the latter reagent was increased to 4 minutes as described in Method B. This method gives results which compare very favorably with those o Method A, and has a marked advantage in simplicity and rapid ity where only the volume per cent olefin is desired. Howeve Method A should be used where additional work is to be done o the unreacted portion of the sample, such as determination of aro matic content, density, or refractive index.

Cooperative results obtained by Sub-Committee XXV o A.S.T.M. Committee D-2 on a considerable number of syntheti and natural samples, using both Methods A and B, will appear i a later paper.

ACKNOWLEDGMENT

The author wishes to express his thanks to A. H. A. Heyn o the Sun Oil Co. for the suggested design of adapter, which make more convenient observation of the point at which the oily dis tillate in Method A becomes heavier than water.

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Analysis of Furfural-Water Solutions

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Rapid and accurate analyses have been developed for determination of water in furfural by a cloud-point method, and for furfural in water by an electrometric titration with bromine.

SIMPLE, dependable analyses of furfural-water solutions are needed in connection with furfural solvent refining and extractive distillation processes. Current methods for analyzing water in the furfural phase include titration with the Karl Fischer reagent, toluene distillation, and centrifuge, refractive index, and cloud-point (saturation temperature) determinations. Methods for analyzing furfural in the water phase include ultraviolet absorption (spectrometric), colorimetric determinations with fuchsin-sulfite and with aniline reagents, titration with hydroxylamine hydrochloride, and gravimetric determinations with special reagents such as phloroglucinol. None of the above methods has superseded the others to the extent of being specified for standard or reference analyses. The methods given here are based on calibrations of known quantities of furfural and of water, and are of sufficient speed and accuracy for general use.

Water in furfural is determined by observation of the cloud point of equal volumes of furfural and a cloud-point reagent consisting of a mixture of 1-hexanol and cottonseed oil. Cloud points with cottonseed oil alone occur at undesirably high temperatures, and are lowered by the presence of the alcohol; by adjusting the ratio of oil to alcohol, the cloud point may be brought to an optimum temperature level.

This procedure and the cloud-point behavior resemble the determination of water in aniline with the cottonseed oil–mineral oil reagent developed by Seaman, Norton, and Hugonet (*5*). Using dry furfural, the method should be adaptable to the determination of water in any other organic liquid that does not react with furfural merely by substituting it for 1-hexanol.

Hughes and Acree (*3*, *4*) reported that furfural reacts with bromine to equimolar proportions rapidly and with a second mole or more of bromine slowly at 20° to 30° C. The first mole of bromine (reacted at 0° C.) yielded 2 moles of hydrogen bromide. The same authors developed a satisfactory bromometri analysis for furfural that consists essentially of providing exces bromine with bromide-bromate reagent, reacting at 0° C. in a 3% hydrochloric acid solution, replacing the excess bromine with iodine using potassium iodide, and then titrating the free iodin with standard thiosulfate and starch indicator. The metho specifies 0.1 *N* reagents and is not well adapted to samples con taining only a few milligrams of furfural.

In this laboratory, attempts at direct titration of furfura using bromide-bromate reagent showed that the rate of the firs

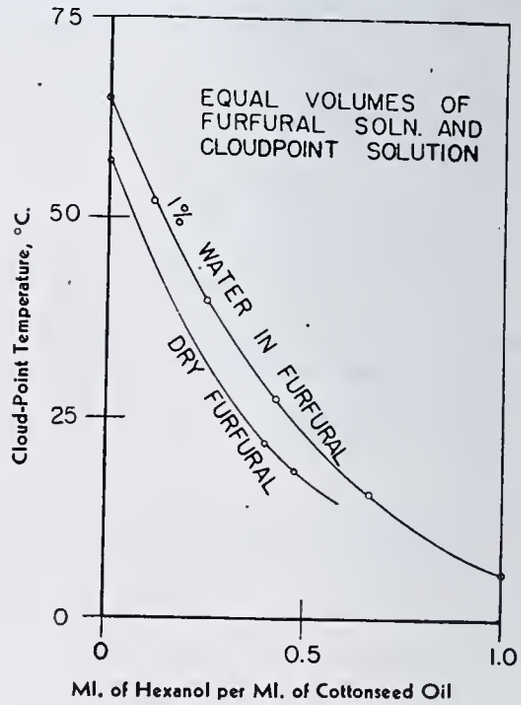

Figure 1. Effect of Hexanol–Cottonseed Oil Ratio on Cloud Point

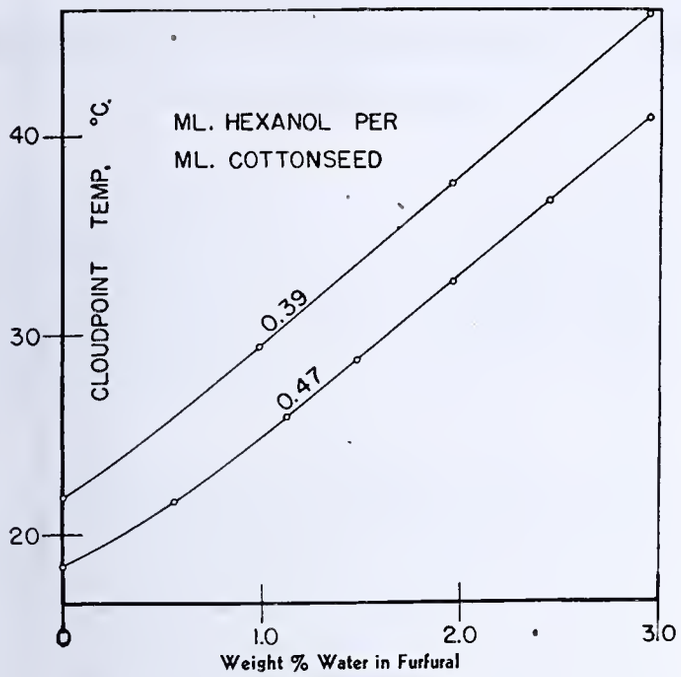

Figure 2. Effect of Water Concentration on Cloud Point of Furfural Solutions with Hexanol–Cottonseed Oil Mixtures

reaction was far too slow to be satisfactory, but that in the presence of hydrochloric acid and mercuric chloride, free bromine in aqueous solution reacts rapidly as far as equimolar proportions. This permits observation of an end point when free bromine persists in the solution. The end point is conveniently observed as e.m.f. (or pH) of a calomel electrode. The e.m.f. rises sharply from about 250 to over 800 millivolts (0.25 to 0.8 volt) and persists for a limited time when the end point is reached. A certain excess of bromine is consumed over the stoichiometric equivalent. But with proper precautions and attention to detail, the end point is reproducible. Plots of stoichiometric bromine against bromine to "potentiometric end points" gave straight lines for any given concentration of bromine water, so the reagent may be standardized by titration against pure furfural in terms of its "potentiometric normality".

CLOUD-POINT DETERMINATION OF WATER

Materials and Equipment. The 1-hexanol may be obtained from Carbide and Carbon Chemicals Corp., and is dried by distilling off the water before use. The cottonseed oil may be commercial Wesson oil. The furfural may be obtained from Quaker Oats Co. and is dried and purified by redistillation under reduced pressure, rejecting the first 25% of distillate.

The cloud-point apparatus is a 2.5 × 10 cm. (1 × 4 inch) test tube mounted in a water bath consisting of a 250-ml. beaker warmed by a Bunsen burner. The test tube contains an A.S.T.M. titer test thermometer extending to about 1.25 cm. from the bottom. A wire bent to form a loop encircling the thermometer is used as the stirrer. The thermometer is mounted in a cork with an off-center hole for the wire stirrer. The A.S.T.M. aniline point apparatus (*1*) may be used with equal satisfaction.

Procedure. The cloud-point reagent is made up of about 0.4 ml. of dry hexanol per ml. of cottonseed oil and is kept in a glass-stoppered flask.

The cloud-point apparatus is charged with 10 ml. of furfural and 10 ml. of cloud-point reagent. The mixture is warmed with stirring until it is perfectly clear and transparent, then cooled slowly with stirring until the cloud point is observed. A haze appears as the cloud point is approached, and the solution becomes suddenly opaque at about 0.75° C. lower. The "opaque point" is reproducible to ±0.1° C., and is taken as the cloud point. The reagents are calibrated against samples of wet furfural made up with known amounts of water. Sample calibration tests are plotted in Figures 1 and 2.

It is recommended that samples containing more than 3% water be mixed with 1, 2, or 3 parts of dry furfural for testing. For samples containing less than 1% water, a slight improvement in accuracy and convenience is obtained by using a cloud-point reagent of about 0.3 ml. of hexanol per ml. of cottonseed oil.

BROMOMETRIC TITRATION OF FURFURAL

Materials and Equipment. The titration cell consists of a 250-ml. beaker marked at the 100-ml. level and equipped with a stirrer, a thermometer, a normal calomel electrode, and a reference electrode of a spiral of platinum wire sealed in a glass tube (Figure 3). The standard bromine reagent is delivered from a 50-ml. buret.

The cell is mounted in a water bath, such as a 1-liter beaker, since the temperature must be constant to ±1° C. or less during a titration.

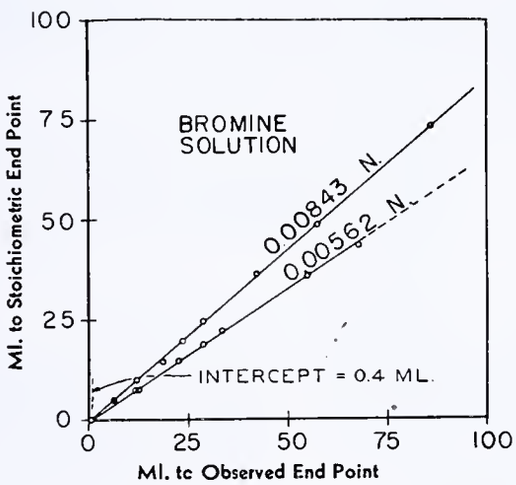

Figure 4. Potentiometric Titration of Furfural in Water at Room Temperature

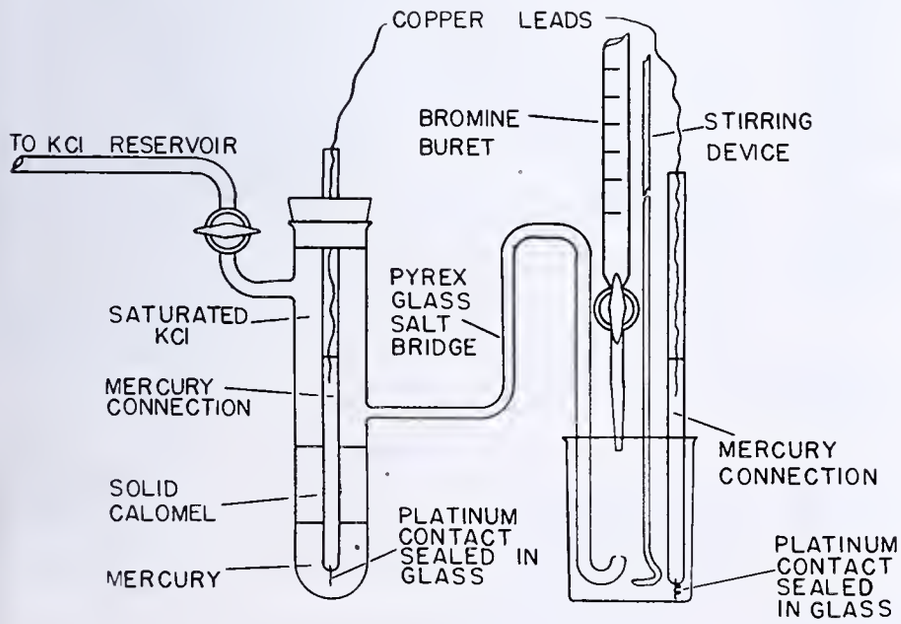

Figure 3. Potentiometric Titration Assembly

The e.m.f. (or pH) is observed by a sensitive potentiometer, an electronic voltmeter, or a pH meter of the proper range (0 to 1 volt), such as is specified for the A.S.T.M. acid and base numbers electrometric titration apparatus (*2*).

The bromine reagent is made up approximately 0.005 molal (0.005 *N*) in bromine, 0.01 molal (0.01 *N*) in potassium bromide, and with 10 ml. of concentrated hydrochloric acid per 100 ml. of distilled water. It should be made up fresh daily and standardized against furfural.

Mercuric chloride as saturated solution in distilled water is run through the electrode into the cell for each titration.

Procedure. The bromine reagent is calibrated against 5-mg. samples of furfural, using the same titration procedure as for unknowns. At any constant temperature, the volume of reagent plots as a straight line against amount of furfural with an intercept of −0.4 ml. of 0.005 *N*

bromine (Figure 4). After subtraction of 0.4 ml. from the buret reading at the end point, a "potentiometric normality" of the reagent may be used (sample calculations).

A "temperature factor" read from Figure 5 is used when the bromine calibration is made at a different temperature from that at which samples are tested.

In general, two titrations are run on an unknown sample, the first to determine the approximate furfural concentration, and the second on a size of sample that contains approximately 5 mg. of furfural.

For the second titration, the titration beaker is charged with the sample and diluted with distilled water to the 100-ml. mark; 3 ml. of the mercuric chloride solution are added and the stirrer is started. When the temperature is constant, bromine reagent equivalent to about 95% of the end point is run in from the buret as fast as it will drain, or within 60 seconds. This will cause a temporary or false end-point potential. As soon as the e.m.f. has fallen below 0.75 volt, bromine reagent is added in increments of approximately 1% of the expected total until the e.m.f. remains above 750 millivolts for 90 seconds. This is taken as the end point.

TITRATION OF FURFURAL IN WATER. SAMPLE CALCULATIONS.

Molecular weight of furfural = 96.1

Specific gravity of aqueous solutions = 1.00

Standardization of bromine reagent: 1.000 gram of dry furfural made up to 1 liter with distilled water. 1 ml. = 1 mg. of furfural.

Titrate 5.0 ml. with bromine reagent. 17.0 ml. required at 21° C. From Figure 5, temperature factor = 1.040.

Potentiometric normality

$$\text{Normality factor} = \frac{(\text{mg. of furfural})\ (\text{temperature factor})}{(\text{molecular weight})\ (\text{ml.} - \text{zero correction})}$$

$$= \frac{(5.0)\ (1.040)}{(96.1)\ (17.0 - 0.4)} = 0.00326$$

TITRATION OF SAMPLE. For second (final) titration of an unknown, take a size of sample that will consume approximately the same volume of bromine reagent as required for the standardization.

20.0 ml. of sample

18.8 ml. of reagent required to end point at 28° C.

Temperature factor from Figure 5 = 1.115

Weight % of furfural

$$= \frac{100\ (\text{ml.} - \text{zero correction})\ (\text{n.f.})\ (96.1)}{1000\ (\text{ml. of sample})\ (\text{temperature factor})}$$

$$= \frac{(18.8 - 0.4)\ (0.00326)\ (9.61)}{(20.0)\ (1.115)} = 0.0259$$

ACCURACY AND APPLICABILITY

WATER IN FURFURAL. The slope of the curves of Figure 2 is about 8° C. for 1% water. A cloud point accurate to ±0.1° C. then gives a reproducibility of ±0.01% water. The cloud point is not sensitive to changes in the proportion of sample to cloud-point reagent. While clean, redistilled furfural was used in the development of the method, polymer or decomposition products, acidity, and hydrocarbons present in plant samples may affect the cloud point. For determinations on impure samples, it is recommended that calibrations of the cloud-point reagent on pure furfural be checked against a calibration using dehydrated plant furfural to avoid possible error from impurities.

FURFURAL IN WATER. As discussed earlier, the bromine reaction is neither instantaneous nor does it stop completely with the consumption of the first mole of bromine per mole of furfural. Furthermore, aqueous bromine tends to hydrolyze according to the equation

$$Br_2 + H_2O \rightleftharpoons HBr + HBrO$$

To avoid errors introduced by the above phenomena, conditions must be rigidly standardized in the respects noted in the procedure.

The use of hydrochloric acid, potassium bromide, and mercuric chloride has two effects: The first two compounds stabilize the bromine reagent, greatly reducing the rate of hydrolysis. Hydrolyzed material does not give reproducible end points; hence the reagent must be made up fresh daily. All three halides appear to act catalytically, speeding the reaction of the first mole of bromine without a corresponding effect on further bromine consumption. The end point is not appreciably affected by wide variations in acid or salt concentrations.

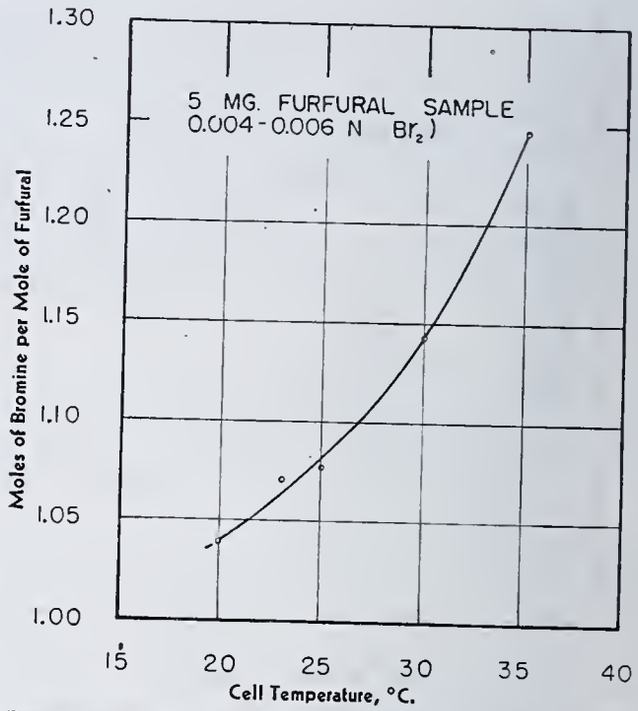

Figure 5. Effect of Temperature on Bromine Requirement

Electrometric titration of furfural in water

The bromine required for the end point depends upon the rate of bromine addition, and to a great extent upon temperature. The use of the temperature factor (Figure 5) may be eliminated and a slight improvement of accuracy gained by thermostating the water bath, so that bromine standardization and the determinations are both made at the same temperature. The method will obviously give high results on a sample containing impurities that react with bromine.

Bromine concentration of 0.005 *N* and sample size of 5 mg. of furfural were selected somewhat arbitrarily for accuracy and convenience. With proper care and attention to detail, a titration is reproducible to ±0.5% of the total furfural in a 5-mg. sample. The absolute accuracy as per cent furfural in water then depends upon the concentration and sample size:

Furfural Concentration %	Sample (Containing 5 Mg. of Furfural) Ml.	Accuracy % *Furfural in water*
0.01	50	±0.00005
0.1	5	0.0005
1.0	0.5	0.005
5.0	0.1	0.02

The above degree of accuracy is not attainable when the sample contains an amount of furfural appreciably different from the amount used in standardizing the bromine reagent. While the accuracy relation is incompletely known, a sample containing either 4 or 6 mg. of furfural titrated with reagent standardized against 5 mg. of furfural gives an accuracy of about ±1.0% of the total furfural present.

ACKNOWLEDGMENT

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Solubility of Magnesium Ammonium Phosphate Hexahydrate

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Despite the appreciable solubility of magnesium ammonium phosphate hexahydrate in solutions of electrolytes, the composition of the solution of analysis is such as to render the salt almost completely insoluble therein. If the recommended procedure for the gravimetric determination of magnesium is employed, recovery as magnesium ammonium phosphate will be complete well within the limits of experimental error.

DESPITE its universal application as the basis of the accepted quantitative analytical procedures for the determination of magnesium (*4*) and phosphorus (*10*), magnesium ammonium phosphate hexahydrate is widely recognized as being one of the most soluble precipitates extensively used in gravimetric determinations (*5*, *21*). A number of investigators (*1*, *3*, *15*, *19*, *20*) have reported solubility data for magnesium ammonium phosphate, but a comparison of the various data reveals a definite lack of agreement among the published results. Further, the effect of the presence of ammonium hydroxide on the solubility of magnesium ammonium phosphate in solutions containing various added salts has received but little attention. This latter is of the greatest practical importance, since the standard analytical procedures call for the addition of considerable quantities of ammonium hydroxide to the solution of analysis.

This contradictory and incomplete nature of the existing data reflects an obvious need for an accurate and comprehensive determination of the solubility of magnesium ammonium phosphate in various concentrations of aqueous and ammoniacal salt solutions likely to be encountered in analytical practice. The present investigation was undertaken to provide this necessary information.

PREPARATION OF SATURATED SOLUTIONS

Magnesium ammonium phosphate hexahydrate was prepared by the reaction of diammonium phosphate and magnesium chloride in aqueous solution. The precipitate was thoroughly washed with distilled water, anhydrous ethanol, and anhydrous ether. Analysis by igniting weighed samples to magnesium pyrophosphate in tared silica crucibles at 1000° to 1050° C. in a muffle furnace indicated an average purity of 99.88%.

Standard solutions of the various salts in which the solubility of magnesium ammonium phosphate was to be determined were prepared in the accepted manner of diluting a weighed quantity of the salt, the highest grade reagent of commerce, to a definite volume in volumetric equipment of proved accuracy. In many cases, the solution medium for these standard salt solutions was ammonium hydroxide of known normality, prepared by dilution of the concentrated C.P. reagent and checked by analysis.

The saturated solutions of magnesium ammonium phosphate hexahydrate were prepared by agitating 1 gram of the hexahydrate in 200 ml. of the required salt solution for 24 hours in a flask immersed in a constant-temperature bath at 25° ± 0.05° C. At the end of this period, the solution was rapidly filtered, and the filtrate set aside in a tightly stoppered bottle for analysis. A sample of the salt solution before saturation was also set aside for use as a blank.

ANALYSIS OF SATURATED SOLUTIONS

This comprehensive investigation of solubilities, involving a large number of magnesium determinations in solutions whose magnesium content would necessarily be very low, demanded a method of analysis which would allow the rapid, as well as accurate, estimation of very small quantities of magnesium. Colorimetric methods were considered as being most likely to satisfy the requirements of speed and precision.

A number of reagents are known to give color reactions which are characteristic for magnesium (*2*, *6*, *9*, *11*, *12*). Of these, titan yellow has probably received the most intensive study, and it was evident from a careful consideration of the literature references describing colorimetric methods for the determination of magnesium (*7*, *14*, *16*, *18*) that titan yellow offered an excellent possibility of successful application in the present investigation. When magnesium hydroxide is precipitated in the presence of titan yellow by sodium hydroxide, the yellow color of the reagent changes to red or orange red at a pH of 12.5. According to Ludwig and Johnson (*14*), the lake which is formed with dilute magnesium solutions remains dispersed for rather long periods, particularly in the presence of protective colloids such as starch, agar, or dextrose. In the absence of such materials, flocculation occurs readily and the results are not reproducible. Gillam (*7*) advised the addition of hydroxylamine hydrochloride to retard the fading of the color produced, an observation which was fully substantiated in the present work. Stross (*18*) emphasized the necessity of rigid adherence to an established technique, especially during the development of color, if reproducible results are to be obtained. The magnesium–titan yellow complex appears to be of colloid nature, and it is well known that the properties of colloids are influenced by the conditions of their formation.

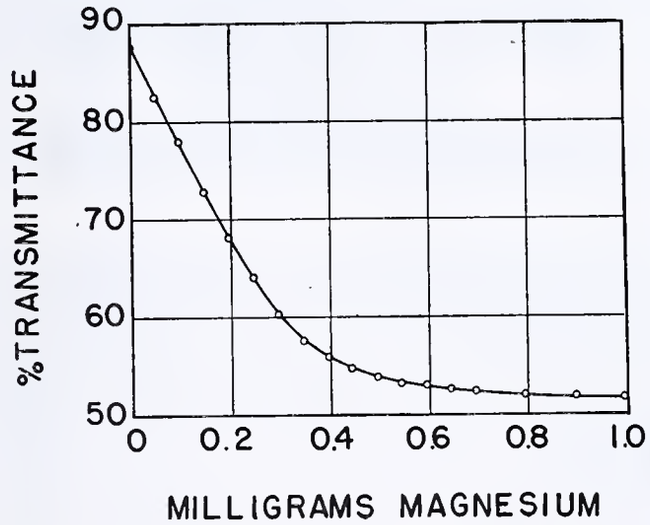

Figure 1. Per Cent Transmittance Versus Magnesium Content of Solution in Which Magnesium–Titan Yellow Complex Has Been Developed

After considerable preliminary experimentation, the colorimetric method developed for use in the present investigation was substantially that proposed by Gillam (*7*):

A carefully measured volume of the saturated solution of magnesium ammonium phosphate was pipetted into a 100-ml. volumetric flask. The sample should contain a maximum of 0.3 mg. of magnesium. This permits a transmittance of at least 60% through the colored solution subsequently developed, as measured in a filter photometer of standard design, employing light filters allowing maximum transmittance of light of a wave length of 524 millimicrons—the approximate region of maximum absorption of light by the color complex. The reason for this limit of sample size is at once apparent upon consideration of Figure 1. Up to a magnesium content of about 0.3 mg., the curve is practically linear and of steep slope. This agrees well with the experience of others (*7*, *8*, *13*, *18*).

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To the sample were then added 10.0 ml. of a 1.0% aqueous solution of gelatin (Eastman Kodak No. 1099). This will effectively prevent flocculation in solutions containing as high as 1.0 mg. of magnesium.

Next, 5.0 ml. of a 4.0% aqueous solution of hydroxylamine hydrochloride (Merck reagent) were added, followed by dilution to approximately 70 ml. with distilled water. The effectiveness of this quantity of hydroxylamine hydrochloride in preventing the fading of the color complex for a period of time sufficient to permit its quantitative determination in the filter photometer is graphically shown in Figure 2.

One milliliter of dye solution (0.15 gram of Eastman Kodak No. P4454 titan yellow in 75 ml. of 95% ethanol and 25 ml. of distilled water) was then added.

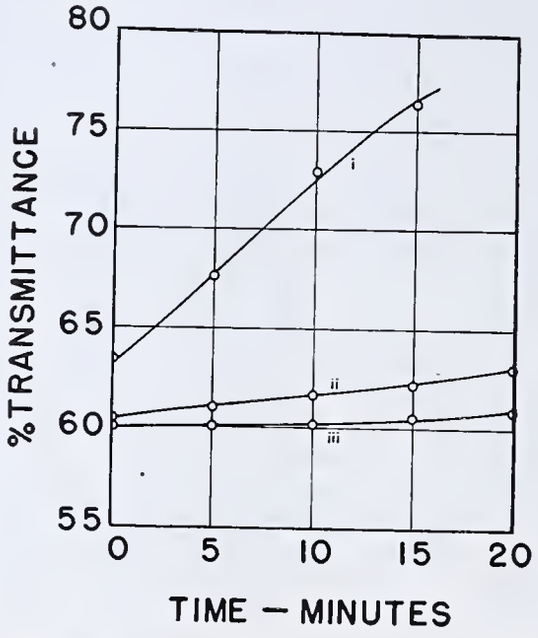

Figure 2. Stabilizing Effect of Hydroxylamine Hydrochloride on Magnesium–Titan Yellow Color Complex Developed in Sample Containing 0.3 Mg. of Magnesium

i. No hydroxylamine hydrochloride added
ii. 2.0 ml. of 4.0% hydroxylamine hydrochloride solution added
iii. 5.0 ml. of 4.0% hydroxylamine hydrochloride solution added

Then 10.0 ml. of a 4.0% aqueous solution of sodium hydroxide (Baker's analyzed) were added with constant swirling, and the solution was made to volume with distilled water and vigorously shaken. If ammoniacal solutions, or solutions containing ammonium salts, were under consideration, a larger quantity of sodium hydroxide solution was required to compensate for the buffering action of the ammonium ion. In either case, the characteristic red color developed immediately in the presence of magnesium.

The per cent transmittance of light of the colored solution thus prepared was measured in the filter photometer.

In this manner, three analyses were made of each of the duplicate saturated solutions of magnesium ammonium phosphate hexahydrate in the specified solution of electrolyte, thus giving six replicate analyses for each solution investigated. The arithmetic mean of the six photometer readings was calculated, and its equivalent in terms of milligrams of magnesium determined by means of a calibration curve. This curve was prepared by developing the magnesium–titan yellow color complex in solutions of the given electrolyte to which had been added measured quantities of a standard magnesium sulfate solution, determining the per cent transmittance of light, and averaging the results for each magnesium concentration to allow a graphical representation of milligrams of magnesium versus photometer reading. It was not found advisable to construct a single calibration curve which would apply throughout the entire investigation. The use of freshly prepared reagents, particularly the gelatin solution, and the amount of caustic solution used to develop the color complex, contributed to slight displacements of the photometer readings for a given magnesium concentration. It was found expedient to analyze all the samples relating to a given added electrolyte in one continuous series of operations and to collect the data required for a calibration curve at the same time.

SOLUBILITY DATA

In Tables I, II, III, and IV, the solubility data are recorded in terms of milligrams of magnesium per liter of solution at 25° C. The data have been grouped in general relation to the magnitude of the normality ranges of the added electrolytes and in several instances are repeated for greater ease of comparison. The data were submitted to a statistical analysis which revealed that in the majority of cases the standard deviation of the average solubility determined for each solution amounted to less than 1% of the average solubility value.

In water, the solubility of magnesium ammonium phosphate hexahydrate was found to be equivalent to 13.6 mg. of magnesium per liter of solution at 25° C.

Selections of solubility curves, representing the extreme effects of added electrolytes, are presented in Figures 3 and 4. These allow a convenient orientation of the relative solubilities of magnesium ammonium phosphate in the several solution media investigated.

Table I. Solubility of Magnesium Ammonium Phosphate Hexahydrate

Salt Solution	Normality of Salt Solution 0.001	0.005	0.010	0.025	0.050	0.075	0.100
	Mg. of magnesium per liter of solution						
NH_4OH	9.60	3.42	1.39	...	0.70	...	0.53
$(NH_4)_2HPO_4$	12.7	8.10	4.68	2.29	1.26	1.12	1.00
Na_2HPO_4	13.1	9.93	8.95	...	8.14	...	7.66

Table II. Solubility of Magnesium Ammonium Phosphate Hexahydrate

Salt Solution	Normality of Salt Solution 0.05	0.10	0.20	0.30	0.40	0.50
	Mg. of magnesium per liter of solution					
$(NH_4)_2C_2O_4$						
Aqueous solution	38.8	57.0	89.5	121.0	152.0	180.0
In 0.01 *N* NH_4OH	22.5	...	64.5	...	112.0	...
In 0.10 *N* NH_4OH	8.76	...	27.1	...	47.7	...
In 1.00 *N* NH_4OH	2.63	...	8.32	...	14.4	...
$Na_2C_2O_4$						
Aqueous solution	43.5	68.5	106.0	138.0	166.0	191.0
In 0.01 *N* NH_4OH	22.1	...	60.5	...	109.0	...
In 0.10 *N* NH_4OH	6.57	...	21.3	...	39.4	...
In 1.00 *N* NH_4OH	2.40	...	7.00	...	11.9	...
$(NH_4)_2MoO_4$						
Aqueous solution	340.0	493.0	730.0	...	...	...
In 0.05 *N* NH_4OH	...	...	404.0	...	...	...
In 0.20 *N* NH_4OH	...	...	2.5	...	...	...

Table III. Solubility of Magnesium Ammonium Phosphate Hexahydrate

Salt Solution	Normality of Salt Solution 0.10	0.50	1.00
	Mg. of magnesium per liter of solution		
NH_4Cl	19.1	38.0	53.0
NaCl	22.3	32.0	37.0
KCl	18.8	26.4	29.8
NH_4NO_3	19.3	35.0	46.5
$NaNO_3$	20.0	30.5	35.0
KNO_3	17.9	24.6	26.0
$(NH_4)_2SO_4$	24.1	52.2	73.0
Na_2SO_4	25.0	38.6	46.0
K_2SO_4	21.9	34.2	39.2
$CaCl_2$			
Aqueous solution	296	499	622
In 0.1 *N* NH_4OH	131	311	416
In 1.0 *N* NH_4OH	18.3	26.9	27.9
In 2.0 *N* NH_4OH	6.6	12.1	12.9
$BaCl_2$			
Aqueous solution	125	184	199
In 0.1 *N* NH_4OH	70	140	155
In 1.0 *N* NH_4OH	15.3	26.0	27.6

Table IV. Solubility of Magnesium Ammonium Phosphate Hexahydrate

Salt Solution	Normality of Salt Solution 0.10	0.25	0.50	0.75	1.00	1.50	2.00	3.00	5.00
	Mg. magnesium per liter of solution								
NH_4OH	0.53	..	0.44	..	0.55	..	...	0.70	0.80
$(NH_4)_2HPO_4$	1.00	..	0.76	..	0.50	..	...	...	...
NH_4Cl									
Aqueous solution	19.1	25.9	38.0	46.0	53.0	61.5	67.0	79.0	86.0
In 0.01 *N* NH_4OH	7.24	..	16.5	..	22.0	..	30.0	...	...
In 0.10 *N* NH_4OH	2.11	..	5.53	..	7.46	..	9.30	...	...
In 1.00 *N* NH_4OH	1.20	..	2.96	..	4.20	..	5.60	...	...
NH_4NO_3									
Aqueous solution	19.3	24.6	35.0	42.0	46.5	52.0	54.0	56.5	59.0
In 0.01 *N* NH_4OH	6.80	..	14.4	..	19.1	..	26.0	...	...
In 0.10 *N* NH_4OH	1.71	..	4.50	..	5.90	..	7.65	...	...
In 1.00 *N* NH_4OH	1.22	..	2.80	..	4.00	..	5.30	...	...
$(NH_4)_2SO_4$									
Aqueous solution	24.1	35.8	52.2	64.0	73.0	87.0	97.5	119.0	148.0
In 0.01 *N* NH_4OH	9.24	..	22.7	..	35.0	..	56.0	...	...
In 0.10 *N* NH_4OH	3.07	..	8.85	..	13.8	..	21.0	...	...
In 1.00 *N* NH_4OH	1.25	..	3.70	..	5.90	..	10.0	...	...
NaCl									
Aqueous solution	22.3	27.2	32.0	35.0	37.0	39.4	40.2	39.6	36.2
In 0.01 *N* NH_4OH	6.30	..	11.7	..	15.1	..	16.6	...	...
In 0.10 *N* NH_4OH	1.75	..	3.78	..	4.76	..	5.66	...	...
$NaNO_3$									
Aqueous solution	20.0	25.4	30.5	33.4	35.0	37.4	38.2	37.0	29.0
In 0.01 *N* NH_4OH	6.00	..	11.4	..	13.8	..	14.7	...	...
In 0.10 *N* NH_4OH	2.70	..	6.35	..	8.86	..	12.7	...	...
Na_2SO_4									
Aqueous solution	25.0	32.0	38.6	42.8	46.0	51.0	54.0	...	...
In 0.01 *N* NH_4OH	8.53	..	19.6	..	27.4	..	34.5	...	...
In 0.10 *N* NH_4OH	2.70	..	6.35	..	8.86	..	12.7	...	...
In 1.00 *N* NH_4OH	0.80	..	2.40	..	3.64	..	4.80	...	...

SOLUBILITY OF MAGNESIUM AMMONIUM PHOSPHATE IN THE SOLUTION OF ANALYSIS

The quantitative determination of magnesium by precipitation as magnesium ammonium phosphate followed by ignition to magnesium pyrophosphate has been investigated by Epperson (*4*). Analysis of aliquots of a standard solution of magnesium chloride gave closely checking true results even in the presence of large excesses of added salts. In no case was there evidence of loss of magnesium ammonium phosphate by solution.

The problem of the solubility of magnesium ammonium phosphate in the solution of analysis of both phosphate and magnesium was further investigated by Hoffman and Lundell (*10*). In the case of phosphate analysis, they found the loss of magnesium ammonium phosphate by solution to amount to the equivalent of about 0.02 mg. of magnesium for each precipitation. Concerning the solubility of magnesium ammonium phosphate under the conditions which exist in the determination of magnesium by the standard procedure, they state that its direct determination is difficult, but by an indirect method they estimated a loss of 0.01 mg. of magnesium by solution of magnesium ammonium phosphate for each precipitation.

This insignificant solubility of magnesium ammonium phosphate in the solutions of analysis as reported by Hoffman and Lundell was confirmed by the direct determination of the magnesium remaining in solution by the colorimetric method of analysis herein described, after precipitation as magnesium ammonium phosphate by the recommended procedure.

A standard solution of magnesium sulfate heptahydrate (Baker's analyzed) was prepared to contain 12.25 grams of the salt in 2 liters. Aliquots of this stock solution containing the equivalent of 0.2771 gram of magnesium pyrophosphate were analyzed for magnesium by the method of Epperson (*4*). The filtrates and wash liquors of each of the four determinations made were combined, and the volumes were reduced by evaporation, transferred to 100-ml. volumetric flasks, and made to volume with distilled water. Aliquots of each solution were analyzed colorimetrically for magnesium. Further determinations were made in which additions of ammonium salts to the sample solutions preceded the gravimetric analysis. The filtrates and wash liquors were treated as outlined above.

The results of the gravimetric analyses are presented in Table V. It is evident from the close agreement of the actual and theo-

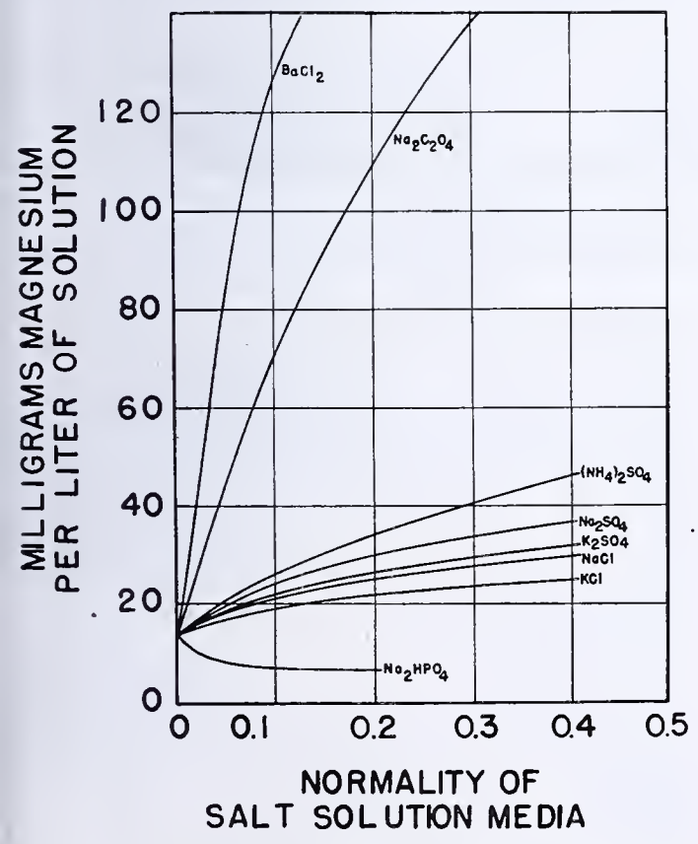

Figure 3. Solubility of Magnesium Ammonium Phosphate Hexahydrate in Salt Solutions

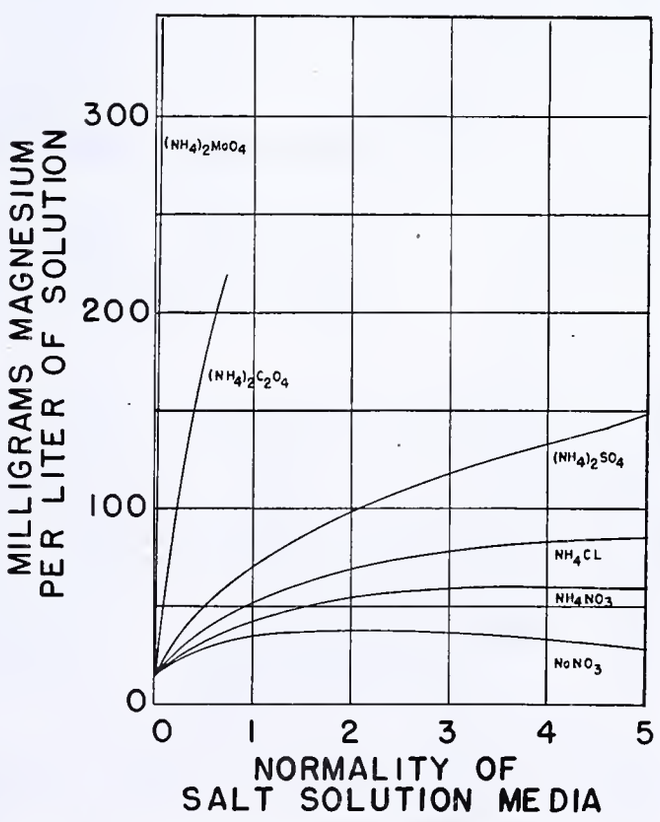

Figure 4. Solubility of Magnesium Ammonium Phosphate Hexahydrate in Salt Solutions

Table V. Gravimetric Analyses for Magnesium

Variation in Standard Procedure	$Mg_2P_2O_7$ or Equivalent Taken	Found
	Gram	*Gram*
No variation	0.2771	0.2772
	0.2771	0.2767
	0.2771	0.2770
Solution of analysis 2.0 *N* with respect to ammonium chloride	0.2771	0.2773
	0.2771	0.2769
	0.2771	0.2771
Solution of analysis 2.0 *N* with respect to ammonium sulfate	0.2771	0.2770
	0.2771	0.2771
	0.2771	0.2766
Solution of analysis 0.3 *N* with respect to ammonium oxalate	0.2771	0.2769
	0.2771	0.2773
	0.2771	0.2769

retical values for magnesium pyrophosphate, that little if any loss of magnesium ammonium phosphate resulted. Further, a statistical analysis of variance (*17*) of these four groups of data indicated that the addition of ammonium salts had no effect on the analytical results.

The photometric analyses of the combined filtrates and wash liquors indicated a maximum magnesium content of 0.003 mg. in the aliquot taken in all cases. The colorimetric method herein described allows the detection of as little as 0.005 mg. of magnesium with a fair degree of accuracy, but below this range the results must be expressed as an estimated maximum. Larger aliquots led to erratic results due to the high salt concentrations in the evaporated solutions. However, the maximum of 0.003 mg. of magnesium in the aliquot taken (10.0 ml.) represents a loss of 0.03 mg. of magnesium in the total filtrate and wash liquors of an analysis involving two precipitations, or approximately 0.015 mg. of magnesium per precipitation. This is in excellent agreement with the value of 0.01 mg. of magnesium per precipitation reported by Hoffman and Lundell.

DISCUSSION OF RESULTS

In general, the presence of added salts effects a considerable increase in the solubility of magnesium ammonium phosphate hexahydrate. Ammonium salts exert a greater influence in th respect than do the corresponding salts of sodium or potassiu in equivalent concentrations. Molybdate and oxalate anio have an unusually high solubilizing effect, indicating the pro able formation of complex ions. The cations calcium and bariu also exhibit exceptional behavior.

However, the addition of relatively small quantities of an monium hydroxide substantially reduced the solubility of ma nesium ammonium phosphate in every salt solution investigate and with increasing additions of ammonium hydroxide the sol bility approached an insignificant figure. This effect is greatest importance in relation to the use of magnesium an monium phosphate as a quantitative gravimetric precipitate analysis.

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Chromatographic Determination of Carotene

Kieselguhr as a Chromatographic Adsorbent

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PHASIC separation of carotene from other pigments by the use of immiscible solvents is now recognized as being unsatisfactory for quantitative separation (*9*). For this reason, chromatographic methods of separating carotene from other pigments present in plant tissues have been increasing in popularity, and a number of methods for determining carotene chromatographically have appeared in the literature in recent years. For the chromatographic determination of total carotenes in routine analysis, an adsorbent should be inexpensive and readily obtained commercially; should be easily packed into the adsorption column; should not contract from the column walls if drawn dry of solvent; should not require special activation or special storage precautions; and should allow a rapid flow of solvent through the column and rapid development of the chromatogram. All noncarotene pigments should be adsorbed and firmly held in the column, while the carotenes should not be appreciably ab sorbed but should wash through the column with the solvent.

Tswett (*15*) introduced methods of analyzing for carotene based on the fact that powdered sugar, calcium carbonate, an inulin do not adsorb carotenes but do adsorb the other plan pigments from a solution of the pigments in petroleum ethe These adsorbents, however, do not meet all the requirement Powdered sugar is reportedly the most satisfactory, lacking onl a high flow rate.

In the search for an adsorbent meeting the above require ments as far as possible, Kernohan (*7*), working in this labora tory, introduced soda ash, the properties of which have recently been described in greater detail by Cooley, Christiansen, an Schroeder (*5*). More recently, various mixtures of Micro Brand magnesium oxide with soda ash or Johns-Manville Hyfl Super-Cel have been introduced which have many of the de sired properties (*4*, *10*, *16*). These mixtures do not, however,

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Heat-treated diatomaceous earths, which have been widely used as filter aids and diluents in preparing adsorbents for chromatographic purposes, possess in themselves a large number of the desired properties of adsorbents for the chromatographic determination of carotene. Hyflo Super-Cel can also be used in the determination of cryptoxanthin and probably in some types of vitamin A analysis. Some methods of extracting carotene from dehydrated alfalfa meal are compared.

heet all requirements, as it is necessary to mix the two materials efore use, and, for the development of the chromatogram, to noisten the adsorbent in the column with petroether, then to add ne solution of plant pigments in petroleum ether and to adsorb ne pigments on the column, and finally to elute the carotenes om the column with a solution of acetone in petroleum ether. Soda ash has the advantages of low cost and of being used ithout mixing or other preliminary treatment. The solution f plant pigments in petroleum ether or similar solvent may be oured directly onto the dry column, and, as soda ash has almost o adsorptive power for carotenes, the carotenes are washed di-ctly through the column with petroleum ether without the ecessity of using special solvent mixtures to elute the carotenes fter adsorption or of separating the pigment-free solvent om the pigment solution, as is required when eluting carotenes om mixtures of magnesium oxide and Hyflo Super-Cel. The dsorption bands of noncarotene pigments are not, however, as harply defined on soda ash chromatographic columns as is sometimes desired. The adsorbent must be protected from atmospheric moisture, and only one technical grade of soda ash, Wyandotte light soda ash, Wyandotte Chemical Corp., Michigan Alkali Division, Wyandotte, Mich.) has been found to have he proper adsorptive properties.

Experiments in this laboratory have shown that heat-treated iceous earths possess to a large degree the requisite properties f an adsorbent for routine determination of carotene, and this dsorbent has been used in this laboratory for the past six years. ne commercial brands of Johns-Manville Hyflo Super-Cel, Celite 1, Celite 535, and Celite 545 have all been found satisfactory r the chromatographic determination of carotene. Hyflo per-Cel is, however, the strongest adsorbent for the carotenoid gments and has therefore been adopted for use in this laboratory. Strain *et al.* (*14*), found that Celite 501 adsorbed the lorophylls and xanthophylls from a petroleum ether solution of ant pigments, and used this heat-treated siliceous earth for e separation of xanthophylls. They found this adsorbent use-l because of the clear separation of pigment bands and the high tration rate of the columns.

Although Hyflo Super-Cel has been widely used as a diluent d filter aid for other chromatographic adsorbents, apparently tle use has been made of its adsorptive properties, though these ve been known for some time (*11*, *18*). Used in an adsorption column in proper amounts, it separates almost all noncarotene pigments from a petroleum ether solution of plant pigments, hile allowing the carotenes to pass through the column almost adsorbed (*17*). Lycopene, however, cannot be separated om carotenes with this adsorbent. Hyflo Super-Cel is inexnsive, uniform in properties, and exceptionally stable. After orage in open sacks for 5 years it was found to be as active as wly manufactured material. Its rapid flow rate and other desirable properties when used in an adsorbent column are well own to workers in this field. As the heat-treated siliceous rths are comparatively weak adsorbents, they are more strongly fected by traces of polar solvents, such as acetone or methanol, an are the mixtures of magnesium oxide with Hyflo Super-Cel.

Preparation of the pigment solution for chromatographing dends on the nature of the material under consideration. Any of the well-known methods of obtaining a solution of plant pigments in hydrocarbon solvent may be used, as indicated by the circumstances. The method of saponification with alcoholic potassium hydroxide and extraction of the pigments from the alcoholic solution with petroleum ether is widely applicable, though this method is generally being superseded by less drastic methods of extraction. When polar solvents are used in the extraction of carotene from the material, special care must be taken to remove entirely the polar solvents from the petroleum ether solution, as they will otherwise elute the adsorbed pigments from the chromatographic column. Hyflo Super-Cel is especially well adapted to the separation of cryptoxanthin from carotene by the method of Buxton (*3*); the cryptoxanthin does not pass through the chromatographic column, but forms a band on the column at some distance from any xanthophylls, thus permitting ready separation and elution of the cryptoxanthin.

PROCEDURE

Extraction of Carotene. In the determination of the carotene content of dehydrated alfalfa meal, the pigment solution may conveniently be prepared by the method of Wall and Kelley (*16*), in which the meal is refluxed for 30 minutes with a mixture of acetone and petroleum ether (Skellysolve B), or by the method of Silker, Schrenk, and King (*10*), in which the finely divided sample is allowed to stand overnight in the dark in a mixture of acetone and Skellysolve B. When low-boiling petroleum ether (boiling range 30° to 60° C.) is used in place of Skellysolve B, a somewhat modified procedure is necessary to remove all the acetone by volatilization prior to chromatographing the solution. Filtration of the sample before heating is, however, unnecessary. The modified procedure used in this case is as follows:

One or 2 grams of finely ground alfalfa meal (preferably through a 40-mesh screen) are weighed into a 125-ml. Erlenmeyer flask and covered with 60 ml. of a mixture of 1 part of redistilled acetone to 2 parts of petroleum ether. The tightly stoppered containers are then set aside in the dark for at least 16 to 18 hours, usually overnight, though an extraction period up to 70 hours may be used if desired. The solutions are decanted into 300-ml. tall-form beakers, the flasks are rinsed twice with two 50-ml. portions of petroleum ether, the beakers are placed on the water bath, and the solutions are evaporated to a volume of 10 to 15 ml. The solutions must not be allowed to evaporate to dryness, or loss of carotene will occur. Fifty milliliters of fresh petroleum ether are then added to the beakers, and the solution is again evaporated to a volume of 10 to 15 ml. Forty milliliters of fresh petroleum ether are added, and the solution is ready for chromatographing. The entire procedure of removing the acetone requires about 15 minutes.

For routine control work, the method of Kernohan (*7*) may often be of use. A 1-gram sample of finely ground alfalfa meal (passes a 40-mesh screen) is weighed into a 125-ml. Erlenmeyer flask, 100 ml. of low boiling petroleum ether are added, and the flask is tightly stoppered and allowed to stand in the dark for at least 16 hours, though it may be allowed to stand for as long as 70 hours without changing the results.

The solution is immediately ready for chromatographing after standing the proper length of time. Results by this method are somewhat lower than by the methods given above, as explained below.

The solution of pigments may be prepared by other methods for materials to which these methods cannot be applied, depending on the nature of the material. Crude carotene solutions obtained by the method of the Association of Official Agricultural Chemists (*1*) or by similar methods may be purified chromatographically. Care must be taken to wash all traces of alcohol from the solution of the pigments.

Chromatographic Separation of Carotene. The chromatographic column is prepared by packing the adsorbent (Hyflo Super-Cel) into a glass column 3.3 cm. in diameter to a height of from 15 to 25 cm. The adsorbent packs readily into the tube. When only total carotenes are to be determined, it is convenient to pack the column by placing a plug of cotton or

glass wool in the bottom of the tube and forcing the tube into a container of the adsorbent. When enough adsorbent is in the tube, it is pressed firmly down into the tube with a plunger. This method of preparing the adsorption column is unusually rapid and gives sufficiently uniform packing to permit separation of the carotenes from the noncarotene pigments. When it is desired to remove some of the adsorbed pigment bands, such as cryptoxanthin or vitamin A, from the column more uniform packing of the adsorbent into the tube may be obtained by adding successive small portions of the adsorbent to the tube, and pressing the material firmly and smoothly into place with a plunger.

The chromatogram is developed by placing the column under suction and pouring the solution of the pigments, including the sample in the case of dehydrated alfalfa meals extracted by the methods of Silker, Schrenk, and King or of Kernohan, onto the top of the column, allowing the solution to be drawn down to the top of the column, and washing the carotene through the column with fresh petroleum ether until the petroleum ether comes out colorless. It is unnecessary to filter the sample from the petroleum ether solution, as the adsorbent in the column acts as an excellent rapid filter. The carotenes pass through the column with the petroleum ether, are removed from the receiving vessel, and are made up to a volume of 200 ml., and the concentration of carotene is determined colorimetrically. A photoelectric colorimeter is used in this laboratory. The total adsorptive power of the column can be varied by varying the amount of adsorbent in the column.

When dehydrated alfalfa meal is analyzed by this method, most of the noncarotene pigments are adsorbed in the first 1 or 2 inches of the column. The additional length of the column is used to separate certain noncarotene pigments which are occasionally present in dehydrated alfalfa and are weakly adsorbed. These weakly adsorbed pigments behave as carotene in the immiscible solvents method (*1*, *2*). When appreciable quantities of fats are present in the solution, the noncarotene pigments are less adsorbed than normally, and the longer column is required to give complete separation of carotene.

DISCUSSION

Hyflo Super-Cel used in the method outlined above readily separates carotene from the noncarotene pigments which are separated from the carotenes by the immiscible solvents method. It also separates carotene from cryptoxanthin, xanthophyll esters, and acid decomposition products of xanthophylls, none of which are extracted from petroleum ether by 90% aqueous methanol, although saponification transforms the xanthophyll esters to the free xanthophyll, which is extracted by the 90% aqueous methanol. Hyflo Super-Cel is useful for the determination of carotene in ensilage, but cannot be used to determine carotene in tomatoes as it does not separate the carotenes from lycopene. If a petroleum ether solution of crude carotene is prepared from tomatoes by the immiscible solvents method (*1*, *2*), and the pigment solution is poured upon a column of Hyflo Super-Cel 3.3 cm. in diameter by 60 cm. in length, and washed with fresh petroleum ether, an upper red band of lycopene will separate from a lower band of carotene, both pigments being slightly adsorbed. However, the bands are too close together to permit quantitative separation of the pigments. The adsorptive power of different lots of Hyflo Super-Cel varies slightly. In most instances, Hyflo Super-Cel will slightly adsorb carotene, and the first few milliliters of petroleum ether passing through the column are colorless. In some lots, however, there is no indication of any tendency to adsorb carotene.

Hyflo Super-Cel has only slight adsorptive power for vitamin A esters, and so may be used in place of soda ash for the determination of vitamin A in mixed feeds by the method of Cooley, Christiansen, and Schroeder. Strain (*12*) has observed that vitamin A is moderately adsorbed on Celite 501. A similar condition exists with Hyflo Super-Cel. The action of Hyflo Super-Cel on vitamin A and vitamin A esters is shown by the following experiments:

A 0.1-ml. portion of fish oil containing approximately 150,000 I.U. of vitamin A per gram was dissolved in 15 ml. of petroleum ether. This solution was adsorbed in a column of Hyflo Super-Cel 3.3 cm. in diameter by 25 cm. in length, and the column washed with petroleum ether. When the first drops of petroleum ether passed through the column, the column was examined by an ultraviolet light, and showed a narrow, green-fluorescing band 2 to 3 cm. from the bottom of the column. The first portion of the petroleum ether passing through the column was tested with Superfiltrol, and failed to give the characteristic color reaction for vitamin A (*8*, *19*). The adsorbent was removed from the tube and the portion containing the fluorescing band cut from the column. This portion when added to Super-Filtrol with additional petroleum ether gave a strong blue color indicative of the presence of vitamin A. This is presumably vitamin A ester as it is known that vitamin A exists in fish oils primarily as the ester. Repetition of this test, with continued washing of the column with petroleum ether, showed that the vitamin A ester was rapidly washed through the column with petroleum ether.

One milliliter of the oil previously used was saponified, and an aliquot approximately equivalent to that used in the previous test was taken and similarly chromatographed on a column of Hyflo Super-Cel. When the first drops of petroleum ether passed through the column, examination by ultraviolet light showed a green-fluorescing band only 6 cm. from the top of the column. This portion of the column gave a positive test for vitamin A with Superfiltrol. A narrow band which fluoresced green was adsorbed near the top of the column, but failed to give a positive test for vitamin A with Superfiltrol.

To test the ability of Hyflo Super-Cel to separate vitamin A from its ester, 0.1 ml. of the oil dissolved in 15 ml. of petroleum ether containing an aliquot of saponified fish oil was chromatographed on a column of Hyflo Super-Cel 3.3 cm. in diameter by 25 cm. long. When the first petroleum ether passed through the column, examination by ultraviolet light showed two distinct green-fluorescing bands, separated from each other by a distance of about 5 cm. The lower band, presumably of vitamin A ester, was readily washed through the column, leaving the free vitamin A in the column. When a mixture of fish oil, saponified fish oil, and carotene was chromatographed on a column of Hyflo Super-Cel 60 cm. long by 3.3 cm. in diameter, it was possible to observe separate bands of vitamin A, vitamin A ester, and carotene. However, the bands of vitamin A ester and carotene were so close together as to prevent ready separation.

Table I. Comparison of Chromatographic and Immiscible Solvents Method of Determining Carotene in Dehydrated Alfalfa

(Hyflo Super-Cel adsorbent)

Sample No.	Brooke, Tyler, Baker Method	BTB Method Followed by Adsorption	Chromatographic Method
	Mg./100 g.	*Mg./100 g.*	*Mg./100 g.*
1	4.9	4.3	5.0
2	4.1	3.6	3.3
3	8.2	7.0	7.1
4	9.7	8.9	9.2
6	14.1	12.0	12.3
7	24.0	21.7	23.0
8	11.0	10.4	10.7

Table II. Comparison of Petroleum Ether and Petroleum Ether–Acetone Extraction Methods, Impure Acetone Solvent

Carotene, Mg. per 100 grams

Sample No.	Petroleum Ether, Super-Cel Column, Extracted 16 Hours	Petroleum Ether, Super-Cel Column, Extracted 64 Hours	Petroleum Ether–Acetone Super-Cel Column, Extracted 16 Hours	Petroleum Ether–Acetone, MgO Super-Cel Column, Extracted 64 Hours
23	7.6 7.6	7.9	6.2 6.7 7.0[a]	1.0 3.7
24	6.0 6.0	6.2	3.9 6.2 5.7[a]	0.8 2.8
25	3.7 3.7	3.5	2.8 2.8 3.9[a]	0.6 1.0
39	22.0 22.0	21.7	16.6 18.4 17.7[a]	6.7 12.3
41	24.1 23.8	24.1	17.7 19.5 19.0[a]	5.4 11.0
42	25.2 25.8	25.8	21.7 19.2 20.7[a]	4.6 11.0

[a] Petroleum ether–acetone extracted samples not filtered before adding with solvent to chromatographic column.

Although no attempt has been made to develop a method for ing these heat-treated siliceous earths for the determination of amin A, the tests described above indicate the possibility of ch methods. A possible mode of application to the determina- n of vitamin A in mixed feeds would be the preparation of a ution of carotene and vitamin A ester by the method of ooley, Christiansen, and Schroeder (*5*), substituting one of e siliceous earths for the soda ash adsorbent in their method, lowed by saponification and separation of the vitamin A and rotene by chromatographing on Hyflo Super-Cel or Celite 1. Because of the speed with which complete development of a romatogram can be carried out with these siliceous earths, the quirement of two chromatographic procedures should not be duly burdensome.

COMPARISON OF EXTRACTION METHODS

The method originally used in this laboratory for the extrac n of carotenes from dehydrated alfalfa meal preparatory to romatographing is that of Kernohan (*7*), described above sults are very reproducible. The results obtained by this thod, using Hyflo Super-Cel as an adsorbent, and by the im- scible solvents method as modified by Brooke, Tyler, and ker (*2*), are compared in Table I.

A number of authors have shown that much of the pigment de- mined as carotene in the immiscible solvents method is without amin A activity. Kemmerer and Fraps (*6*) compared a num- of methods of determining crude carotene, and found sig- icant variations in the amount of crude carotene and in the mount of the components possessing vitamin A activity present the crude carotene solutions prepared by each method. As can seen from Table I, results obtained by the chromatographic thod using the extraction method of Kernohan may be either her or lower than those obtained by the immiscible solvents thod.

According to Silker, Schrenk, and King (*10*), it is not possible to tract all the carotene from finely divided dehydrated alfalfa al by allowing the sample to stand in petroleum ether as ommended by Kernohan (*7*), but all the carotene can be ex- cted by allowing the sample to stand in a mixture of Skelly- ve B and acetone for 16 to 18 hours. No data, however, are en to confirm this. Comparative tests of the two methods, d re-extraction tests on the alfalfa residue remaining after traction with petroleum ether alone by the method of Kerno- n, have confirmed the claim that extraction with petroleum ether alone by the method of Kernohan is incomplete, and that gher results are obtained by extraction with a mixture of petro- m ether and acetone as recommended by Silker, Schrenk, and ng or by Wall and Kelley (*16*).

Experiments were also conducted to determine whether the traction time of 16 to 18 hours used by Silker, Schrenk, and ng (*10*) could be lengthened to 70 hours without loss of caro- e. It was found that this longer extraction time is satis- tory; thus this extraction method can be used even when oratory work is not conducted on the day following the placing the samples in the solvent, if special precautions are taken to sure the purity of the acetone.

In the first series of tests, somewhat higher results were ob- ned in some instances by the extraction method of Silker *et al.* an by the method of Kernohan, but these were found to be due certain impurities which had passed through the adsorption umns. On more thorough chromatographic purification, gher results were obtained with petroleum ether extraction an by extraction for 16 hours at room temperature with a mix- e of 20 ml. of acetone and 40 ml. of petroleum ether. When s test was repeated, allowing the samples to stand in contact th the solvents for 64 hours, similar amounts of carotene as in e 16-hour extraction were found with petroleum ether, but ich lower and erratic results were obtained with the mixed solvents, as shown in Table II. Loss by oxidation rather than incomplete extraction appears to cause the low results by the latter method.

The acetone used in these tests was commercial C.P. acetone. As this seemed the probable source of oxidation, steps were taken to purify the acetone. Distillation of a similar batch of acetone from an ordinary distilling flask showed it to have a boiling range of from 50° to 70° C. The fraction boiling from 56.5° to 57.3° C., about half the total acetone, was taken for all further tests. Strain and Manning (*13*) recommend the addition of 0.5% redistilled dimethyl aniline to solvents used in extracting carotenoids from plant material in order to neutralize plant acids during extractions, to counteract acidity which might otherwise develop upon the adsorption columns, and to minimize pigment oxidation. As dimethyl aniline was not readily available, tests of an antioxidant were conducted by adding 0.200 gram of hydroquinone to some of the samples extracted by mixtures of acetone and petroleum ether.

Table III. Comparison of Hyflo Super-Cel with Magnesia–Hyflo Super-Cel Mixture as Chromatographic Adsorbents

	Carotene, Mg. per 100 Grams					
	Extracted with Petroleum Ether		Extracted with Acetone and Petroleum Ether		Extracted with Acetone, Petroleum Ether, and Hydroquinone	
Sample No.	MgO	Super-Cel	MgO	Super-Cel	MgO	Super-Cel
2	3.0	3.3 3.4 3.2	4.3	4.4 4.4	4.3	....
3	6.7	6.7 6.7	7.6	7.4	7.6 7.0	7.9 7.4
4	8.9	8.9 9.2	9.2	9.2	9.5 8.9	9.2 9.7
5	12.3	12.0 12.7	14.1	12.5	14.1	14.1 14.1
6	12.3	12.3 12.3	13.9	15.5	13.6	13.3 14.1

The use of hydroquinone to diminish oxidation of carotene is well known. Because of its acid properties hydroquinone cannot be considered a complete substitute for dimethyl aniline, and its use would be inadvisable with any but dehydrated materials. As hydroquinone is insoluble in petroleum ether, it precipitates upon evaporation of the acetone, and thus is removed from the solution. If the hydroquinone were not removed, it would tend to elute the adsorbed pigments from the chromatogram, making separation of the carotene from the noncarotene pigments difficult. This effect was somewhat noticeable when the magnesia adsorbent was used and the carotene eluted with a mixture of 4% acetone in petroleum ether.

Some of the results obtained by extracting with mixtures of petroleum ether and acetone containing added hydroquinone as compared to extracting with mixtures lacking the hydroquinone are shown in Tables III and IV. These results are inconclusive, and show no particular advantage to be gained from the use of hydroquinone. Unfortunately, the lot of acetone believed to have caused the oxidation of carotene was entirely used in the preliminary determinations, and further lots of acetone which were not redistilled failed to show low results when used in extracting dehydrated alfalfa meal. The addition of hydroquinone, dimethyl aniline, or other suitable antioxidant should, however, be a useful precaution against oxidation when plant pigment is extracted by allowing plant material to stand in contact with the solvents for a considerable period of time. Redistilled dimethyl aniline as recommended by Strain and Manning should be preferable to hydroquinone, as the dangers of an acid reaction would be lacking.

Tests with the redistilled acetone in the various extraction methods were again conducted (Tables III and IV). These re-

sults show clearly that higher results are obtained with the extraction method of Silker, Schrenk, and King than with the method of Kernohan. Table III also shows that comparable results are obtained using Hyflo Super-Cel as an adsorbent in the place of the more complicated Hyflo Super-Cel–magnesium oxide mixture. Table IV shows the results of tests of other methods of extraction and different times of extraction. The results in Table IV were obtained over a period of approximately 3 weeks, during which time the samples were stored in the dark at room temperature, so that some of the differences in carotene content shown are due to loss of carotene in the samples during storage. In the method of Wall and Kelley (*16*), 1-gram samples of dehydrated alfalfa meal were refluxed for 45 minutes with a mixture of 30 ml. of acetone and 70 ml. of low-boiling petroleum ether. The time of refluxing was 15 minutes more than that recommended by Wall and Kelley, and was increased by this amount to compensate for the lower boiling temperature caused by using low-boiling petroleum ether in place of Skellysolve B in the mixed solvent. Results obtained by this method were very similar to those obtained by the method of Silker, Schrenk, and King.

To test for the completeness of extraction by the method of Kernohan, 2.5-gram samples of dehydrated alfalfa meal were extracted for 20 hours at room temperature with 150 ml. of low-boiling petroleum ether in a 250-ml. Erlenmeyer flask. The samples were filtered off into Gooch crucibles, and thoroughly washed with petroleum ether. The remaining sample was then treated by hot saponification and the carotene content determined by the immiscible solvents method of Brooke, Tyler, and Baker (*2*). A portion of the crude carotene solution so obtained was chromatographed on a column of Hyflo Super-Cel 3.3 cm. in diameter by 15 cm. in height to remove noncarotene pigments.

Table IV shows that carotene was not completely extracted by petroleum ether, thus confirming the results obtained in comparing carotene determinations made by this method with those made with other, more recently developed extraction methods. However, in view of the fact that approximately 10 to 25% of the carotene determined by the various abridged chromatographic methods has no vitamin A activity (*6*), the extraction method of Kernohan may be useful under certain conditions for routine control work on dehydrated alfalfa meals, as it enables rapid estimation of the vitamin A activity of this material, often with an accuracy comparable to other methods of determining the vitamin A activity from the carotene content.

Table IV. Effect of Extraction Methods on Carotene Content of Dehydrated Alfalfa Meals Determined Chromatographically with Hyflo Super-Cel Adsorbent

		Carotene, Mg. per 100 Grams				
		No. 2	No. 3	No. 4	No. 5	No.
1	90 hours with petroleum ether at room temperature	...	6.7	8.9	13.0	14.
		...	6.7	8.9	13.3	14.
2	Petroleum ether, 20 hours at room temperature	3.3	6.7	9.2	12.0	12.
		3.3	6.7	9.2	12.7	12.
3	Acetone–petroleum ether mixture, 20 hours at room temperature	4.4	8.9	9.7	15.2	15.
		4.4	8.2	10.0	15.7	15.
4	Acetone–petroleum ether mixture, 90 hours at room temperature	...	7.6	9.2	14.1	14.
		...	7.6	9.2	14.7	15.
5	Acetone–petroleum ether–hydroquinone, 90 hours at room temperature	...	7.6	9.7	15.2	16.
		...	7.6	9.7	16.3	16.
6	Wall and Kelley method, reflux with acetone–petroleum ether	4.4	8.2	10.0	15.5	15.
		4.4	8.9			
7	Residue from petroleum ether extraction by immiscible solvents method	0.72	0.96	0.80	1.32	1.2
8	Method 7 followed by adsorption on Hyflo Super-Cel	0.32	0.40	0.28	0.80	0.
9	Sum of methods 2 and 7	4.02	7.66	10.0	13.67	13.

EFFECT OF POLAR SOLVENTS

In preliminary experiments in the comparison of the extraction methods of Kernohan and of Silker, Schrenk, and King, using acetone that was not redistilled, the petroleum ether–acetone extraction gave higher results than petroleum ether extraction for the old samples of alfalfa, and slightly lower results for the newly dehydrated samples. During the analysis, part of a gray-colored band was observed to pass into the solution of carotenes obtained by chromatographing the solution from the petroleum ether–acetone extraction. This band had an orange-red fluorescence under the light from a General Electric mercury projector spotlight which was equipped with a filter to transmit primarily ultraviolet light. Comparison of the appearance under this light of the carotene solutions prepared by chromatographing the solutions from the petroleum ether extraction and the petroleum ether–acetone extraction showed that the latter had a faint reddish fluorescence not present in the solution prepared from samples extracted by petroleum ether alone.

To analyze solutions obtained by extraction with the mixed solvents, special care was given to removing all the acetone from the solutions before chromatographing, and the columns of Hyflo Super-Cel used were 25 cm. long. Under these conditions, none of the gray band passed through the column, and, upon following the progress of the bands through the adsorption column with ultraviolet light, it was found that a colorless, orange-red fluorescing band was adsorbed below the gray band and did not pass through the column. The final carotene solutions did not fluoresce red after this treatment of the extract. The colorless band showing the orange-red fluorescence was removed from the chromatographic column and eluted with acetone. The material when concentrated was found to be slightly yellow in color. The pigment was transferred to petroleum ether which was washed free from acetone. It was then found that this material could not be extracted from petroleum ether with 90% aqueous methanol solution.

Further experiments with modifications of the method of removing the acetone from the petroleum ether and acetone mixture led to the development of the method recommended under Procedure. With this method, the fluorescent band remains firmly held near the top of a column of Hyflo Super-Cel only 15 cm. long, and no danger of contamination of the solution of carotene exists. In this case, several extremely narrow bands of yellow pigment often appear below the fluorescent band. It is recommended, however, as a matter of routine precaution, that all carotene solutions prepared when acetone is used as a solvent and Hyflo Super-Cel as an adsorbent be checked for fluorescence.

The effects of a solution of 4% acetone in petroleum ether in eluting carotenoid pigments from a chromatographic column of Hyflo Super-Cel were tested, using a chromatogram which had been developed from a petroleum ether extract of high-grade alfalfa meal. In this instance, all visible and fluorescent pigments were firmly adsorbed in the top 5 cm. of a 25-cm. column. On percolating the petroleum ether–acetone mixture through the column, the chlorophylls and some yellow pigments remained firmly adsorbed in the column, moving downward only slightly. Three bands separated from these strongly adsorbed pigments and moved down the column rapidly: a lower yellow band, an intermediate gray, and an upper yellow. The mixed pigments contained in the two lower bands were separated from the upper yellow band. The mixed pigments of the lower yellow band and the gray band were not extracted from petroleum ether by 90% aqueous methanol solution. The solution fluoresced red in ultraviolet light. The pigment solution was allowed to stand overnight in contact with a methanolic sodium hydroxide solution, the pigment was extracted from the methanol with petroleum ether, and the petroleum ether solution was extracted with 90% methanol. The remaining petroleum ether solution was a clear yellow color, and showed no fluorescence in ultraviolet light. It therefore appears that this pigment is one of the

nterfering pigments in the method of immiscible solvents. The ıpper yellow band from the chromatogram was readily extracted rom petroleum ether solution by 90% aqueous methanol, and vas probably a xanthophyll.

These preliminary experiments indicate that the use of dilute olution of polar solvent in petroleum ether offers a rapid method f separating small quantities of noncarotene pigments having olubility relations similar to those of carotene.

SUMMARY

Heat-treated kieselguhr, especially Johns-Manville Hyflo Super-Cel, is an excellent chromatographic adsorbent for the outine determination of total carotenes, and can also be used in he separation of cryptoxanthin from carotene. Preliminary ests indicate that this adsorbent may be applicable to the sepaation of vitamin A from carotenoids, and so may be useful in the letermination of vitamin A in certain instances.

Tests have confirmed claims that extraction of carotene from lehydrated alfalfa meal with mixtures of petroleum ether and cetone gives more complete extraction than extraction by alowing the meal to stand in contact with petroleum ether for 16 ours or more. The latter method, however, gives consistent esults and should frequently be useful for routine control work on lehydrated alfalfa meal.

The chromatographic method described is unusually rapid and equires minimum manipulation, thus reducing chances for error nd enabling easy training of operators. As the adsorbent does not shrink from the walls of a chromatographic column if drawn dry of solvent, it is especially convenient when batteries of chromatographic columns are used.

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Molecular Distillation as a Step in the Chemical Determination of Total and Gamma-Tocopherols

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A METHOD for quantitatively concentrating tocopherols from lipides is much needed in the vitamin E analysis of any natural products. Such a procedure is desirable especially or fats and oils of low tocopherol content, to enable the use of thanol as the solvent in the colorimetric measurement of tocopherols and to avoid the possible inhibiting effect (*7*) of fats on he color development in the Emmerie-Engel reaction. The auors have found that distillation of the extracted lipide under w pressure in a simple molecular still effects quantitative sepaation of as little as 60 micrograms of tocopherols from 1 gram of t. The distillate, usually 15 to 20 mg. in total weight, contains he tocopherols in a sufficiently concentrated form for colorimetc assay purposes. In addition, all of the chlorophyll and much f the carotenoids remain in the residue.

Dam and associates (*2*, *4*) have reported quantitative recovers by molecular distillation of α-tocopherol added to nonsaponiable matter at a level about 3%. Their standardized distillation onditions were 100° C. at 1μ pressure for 30 minutes. However, ver, in the authors' laboratory, in order to avoid saponification it as necessary to distill fats containing only 0.006 to 0.2% tocopols. For this concentration range quantitative separation is hieved in the apparatus described below by distilling at 215° 220° C. at 1μ or less for 30 minutes.

Saponification, which would appear to be the proper procedure r concentrating tocopherols from lipides, has not proved satisctory for fats of low tocopherol content. Tocopherols at levels mparable to those occurring in animal fats—e.g., 60 micrams per gram—have been recovered to the extent of only 60 to 70%. Saponification has been conducted in a closed system under nitrogen, according to directions given by Chipault *et al.* (*1*) with the use of pyrogallol as an antioxidant, or with a minimum of alkali and exposure to heat, without affording full recoveries. Separation by adsorption of tocopherols on alumina has also failed to give quantitative results in preliminary trials. On the other hand, complete recovery has been obtained by the method described here.

EXPERIMENTAL

A diagram of the distillation apparatus is shown in Figure 1.

Procedure. The fat sample, 1 gram or less, is weighed into the aluminum cup, melted if necessary to form a thin film, and placed in the still. Aluminum strips are attached to the cup merely for ease of handling. The sample is degassed by closing the still with a large rubber stopper and reducing the pressure to less than 1μ at room temperature. This minimizes splashing of the sample in the subsequent distillation. The pressure is restored to atmospheric, and without delay the condenser is inserted and the pressure reduced again to 1μ. The condenser is then filled with acetone and dry ice. The surface of the oil in the bath is adjusted level with the bottom of the condenser. The temperature of the bath is raised rapidly to 220° C. and maintained at 215° to 220° C. for 30 minutes while distillation takes place. Then the oil bath is removed and the still is allowed to cool to room temperature under vacuum. The condenser is removed carefully from the still and the distillate washed off quantitatively with chloroform and transferred, by means of evaporation under nitrogen, to a known volume of absolute ethanol. Separate portions of the sample are used for the analyses of total tocopherols and γ-tocopherol.

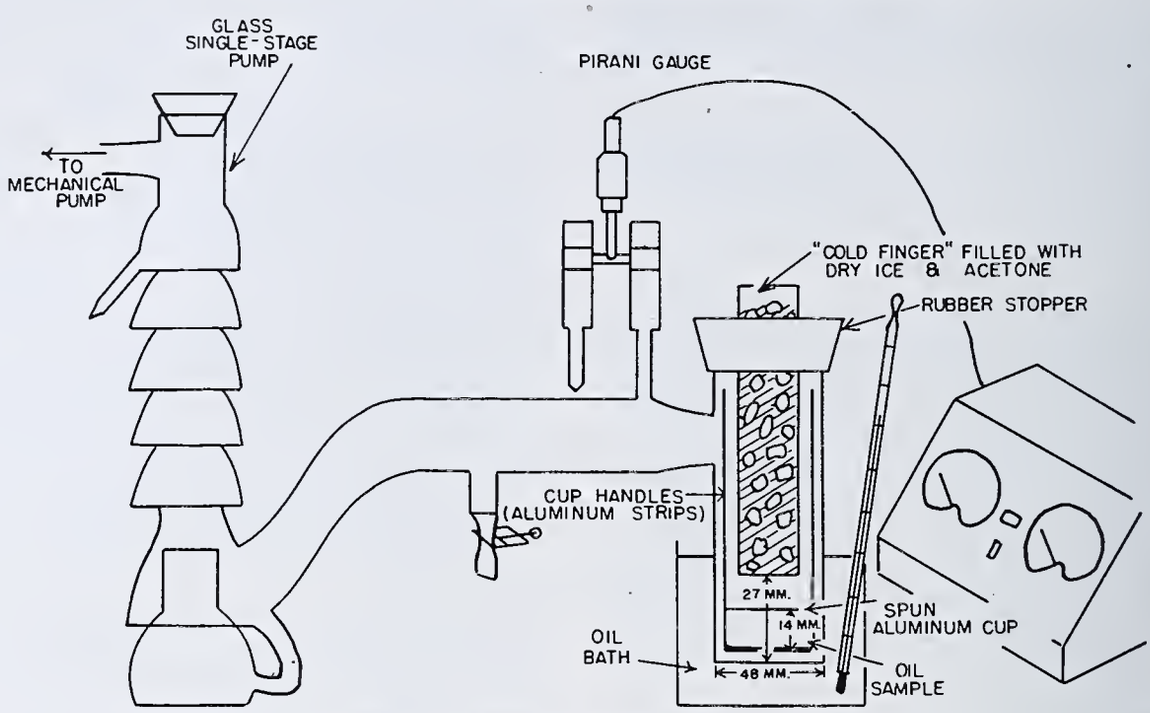

Figure 1. Diagram of Distillation Apparatus

RESULTS AND CONCLUSIONS

Recovery experiments were performed in which γ-tocopherol was added to lard in amounts varying from 0.06 to 2.0 mg. per gram. The base values of the lard, given in Table II, were 27 micrograms per gram of total tocopherols and less than 4 micrograms per gram of γ-tocopherol. No further tocopherols were found with exhaustive distillation for 3 hours or on raising the temperature to 230° C. The values shown in Table I are the results of duplicate distillations and analyses. The range of recovery values is between 92 and 102%. Gamma-tocopherol values are shown because of their specificity; however, Emmerie-Engel results for total tocopherols also show excellent recoveries. Gamma-tocopherol was measured by use of the Quaife method (*8*) as modified by Weisler (*11*). This procedure involves coupling of γ-tocopherol with a diazo compound to form a red dye; α- and β-tocopherols do not react. Total tocopherols were determined colorimetrically by the Emmerie-Engel reaction (*3*) according to the technique previously described (*10*).

The procedure has been applied to a variety of food fats, a representative selection of which is shown in Table II. Distillates in which carotenoids were known to be present were hydrogenated (*9*) before assaying for tocopherols. The lard distillates were found to be free of substances which inhibit color development of the Emmerie-Engel reaction (*7*). The clarified margarine sample was bioassayed (Miss Marion Ludwig performed the bioassay) and found to contain vitamin E activity equivalent to 0.32 mg. of *d*,α-tocopherol per gram. Chemical determination showed 0.54 mg. per gram of total tocopherols, of which 48% is γ-tocopherol and the remainder probably α-tocopherol. This is in good agreement with the bioassay result. Previous findings (*5*, *6*) relative to the sparing action of γ-tocopherol on α-tocopherol in bioassay tests suggest that this mixture of 0.284 mg. of α-tocopherol plus 0.260 mg. of γ-tocopherol would give the same biological response as 0.3 to 0.4 mg. of *d*,α-tocopherol alone.

Experience with the method described leads to the belief that most naturally occurring fats and lipide extracts can be analyzed for tocopherols using the intermediate step of distillation. It is planned to survey common foods for total tocopherol and for γ-tocopherol content and to compare the results with bioassays in selected cases.

Table I. Recovery of γ-Tocopherol by Molecular Distillation

γ-Tocopherol Added to 1 Gram of Lard[a]	Recovery after Distillation
Mg.	%
0.06	92
0.12	95
0.25	101
0.50	99
1.00	93
2.00	102
Av. recovery	97

[a] This contained 27 micrograms per gram of total tocopherols and < 4 micrograms per gram of γ-tocopherol according to distillation analysis.

Table II. Tocopherol Content of Fats Determined on Molecular Distillates of Samples of 1 Gram or Less[a]

Material Distilled	Tocopherol Content Total	Gamma
	γ/gram	
Margarine, clarified[b]	544	260
Lard	27	< 4
Carrot oil (from fresh carrot 0.3% oil)	1,620	0
Cottonseed oil, refined		
Sample 1	726	313
Sample 2	901	345
Egg lipide (from whole egg, 11.5% lipide)	130	44
Olive oil	69	..

[a] No attempt has been made to use samples larger than 1 gram. A larger sample in the present apparatus might form too thick an oil film for molecular distillation. The still could be enlarged to accommodate a larger sample.
[b] Bioassayed for vitamin E activity.

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COMMUNICATION 92 from laboratories of Distillation Products, Inc.

Kjeldahl Determination of Nitrogen

Elimination of the Distillation

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An acidimetric macroprocedure is described for determining nitrogen without a distillation. The ammonium ion is titrated with standard sodium hydroxide in the presence of formaldehyde.

ARTMANN and Skrabal (*1*) and Rupp and Rossler (*7*) have shown that ammonia can be accurately determined by oxidation to nitrogen in alkaline solution by means of standard hypobromite solution, the excess of the latter being determined by adding potassium iodide and acid and titrating the liberated iodine with thiosulfate. Willard and Cake (*8*) and more recently Haanappl (*3*) have used this method to eliminate the distillation in the Kjeldahl determination. This procedure has one serious disadvantage: The alkaline hypobromite solution employed to oxidize the ammonia must be kept between 0° and 5°, since it is unstable at room temperature.

Ammonia reacts rapidly with formaldehyde to form hexamethylenetetramine, which has very weakly basic properties (*6*) ($K_b = 8.0 \times 10^{-10}$). Therefore ammonium salts may be sharply titrated in the presence of formaldehyde with sodium hydroxide and phenolphthalein indicator. Kolthoff (*5*) has found that the reaction between ammonia and an excess of formaldehyde goes so rapidly that the titration may be made directly—i.e., without excess of alkali. This reaction has been employed in the present modification of the Kjeldahl procedure.

The proposed method consists essentially of three steps: (1) The usual destruction of the organic matter by oxidation with hot concentrated sulfuric acid in the presence of potassium sulfate and a mercury catalyst. (2) Neutralization of the excess sulfuric acid with approximately 10 *N* alkali to the methyl red end point. Sodium bromide is present during the neutralization to form a complex and thus prevent the precipitation of mercury compounds.

$$Hg^{++} + 4Br^- \longrightarrow HgBr_4^{--}$$

(3) Titration of the ammonium salt with standard 0.1 *N* sodium hydroxide to the phenolphthalein end point in the presence of formaldehyde.

$$(NH_4)_2SO_4 + 2NaOH \longrightarrow Na_2SO_4 + 2H_2O + 2NH_3$$

$$4NH_3 + 6CH_2O \longrightarrow (CH_2)_6N_4 + 6H_2O$$

REAGENTS

Sodium Hydroxide Solution, 50%. Dissolve 600 grams of sodium hydroxide (Baker's C.P. pellets, 0.004% silica) in 600 ml. of water. Let the solution stand several days in a paraffin-lined rubber-stoppered bottle and decant the clear supernatant liquid.

Standard Sodium Hydroxide Solution, 0.1 *N*. Dilute approximately 8 grams of concentrated (50%) carbonate-free sodium hydroxide solution to 1 liter with equilibrium water. Standardize against potassium biphthalate. Store in an alkali-resistant glass container protected from the atmosphere with an Ascarite tube.

Sodium Hydroxide Solution, approximately 10 *N*. Dilute about 800 grams of concentrated (50%) carbonate-free sodium hydroxide solution to 1 liter with equilibrium water. Store in an alkali-resistant glass container protected from the atmosphere by an Ascarite tube.

Concentrated Sulfuric Acid, C.P., specific gravity 1.84.

Potassium Sulfate Crystals, Baker's C.P.

Methyl Red, 0.004 *M* (0.1%). Dissolve 0.25 gram of indicator in 250 ml. of alcohol.

Phenolphthalein, 0.03 *M* (1%). Dissolve 2.5 grams of indicator in 250 ml. of alcohol.

Neutral Formaldehyde Solution, approximately 18%. Dilute 45 ml. of Merck's reagent-grade formaldehyde (36 to 38%) with an equal volume of water. Add 2 drops of 0.03 *M* phenolphthalein and neutralize with 0.1 *N* sodium hydroxide to the first detectable pink color. This solution should be prepared just before it is used.

Sodium Bromide Solution, 60%. Dissolve 150 grams of Merck's reagent chemical in water and dilute to 250 ml.

Mercuric Oxide, reagent grade.

Eleven organic compounds were purified by repeated crystallization till a constant sharp melting point was obtained. The compounds were dried to constant weight in vacuo in an Abderhalden dryer containing phosphorus pentoxide in the bulb.

PROCEDURE

Weigh accurately a sample that will yield approximately 4 milliequivalents of nitrogen and transfer directly into a 500-ml. 29/42 standard-taper round-bottomed Pyrex flask. This flask is fitted with a 20-cm. (8-inch) removable neck and serves for both digestion and titration. Add 10 grams of anhydrous potassium sulfate, 0.6 to 0.7 gram of mercuric oxide, and 15 ml. of concentrated sulfuric acid. Put the neck of the flask in place, shake the mixture, and heat it in a fume chamber below the boiling point until frothing ceases. Then increase the heat so that the solution boils gently. If necessary, replace the sulfuric acid that has boiled off, but take care that the quantity of acid in the flask at the end of the digestion does not exceed 15 ml. Continue the heating with sulfuric acid until the solution turns colorless and then 20 minutes longer. Cool the flask to at least 50°, carefully add 50 ml. of water, and swirl the flask until the solid material has dissolved completely. Add 10 ml. of 60% sodium bromide, then 2 drops of methyl red. Neutralize the excess sulfuric acid with 10 *N* sodium hydroxide till the methyl red begins to change color. Now boil the solution gently for 3 minutes to expel carbon dioxide, cool to room temperature, and add 10 *N* alkali dropwise till the solution is just yellow. Next add *N* sulfuric acid dropwise till the pink color is restored. Then add the standard 0.1 *N* sodium hydroxide from a buret to the methyl red end point. Read the buret.

Add 30 ml. of 18% formaldehyde solution. At this point, the solution may become slightly pink. Disregard this, and continue the titration till the solution is yellow again. Add 8 drops of phenolphthalein and complete the titration to the first distinct pink color. The alkali used between the methyl red and phenolphthalein end points is equivalent to the nitrogen present.

When more than 17 ml. of concentrated sulfuric acid must be neutralized before titration of the ammonium salt, sufficient silica is introduced as an impurity in the sodium hydroxide to buffer the solution at the phenolphthalein end point and thus prevent a sharp color change. Therefore it is suggested that no more

Table I. Determination of Nitrogen

Compound	% N Theory	Recommended Procedure: No. of determinations	Recommended Procedure: % N mean	Recommended Procedure: Mean deviation	Standard Procedure: No. of determinations	Standard Procedure: % N mean	Standard Procedure: Mean deviation
Acetanilide	10.36	2	10.30	0.03	2	10.33	0.01
Anthranilic acid	10.22	2	10.24	0.02	..	...	...
Benzamide	11.57	2	11.56	0.04	..	...	...
p-Chloroaniline	10.97	3	10.92	0.00	..	...	...
Cysteine	11.57	4	11.53	0.01	..	...	...
Dicyandiamide	66.64	6	66.51	0.01	6	66.54	0.02
p-Dimethylaminobenzaldehyde	9.40	2	9.34	0.01	..	...	...
Diphenylamine	8.28	3	8.23	0.00	4	8.23	0.04
Diphenylcarbamine chloride	6.04	2	6.02	0.01	..	...	...
sym-Diphenylurea	13.21	3	13.17	0.06	..	...	...
Sulfanilic acid	8.08	4	8.07	0.02	..	...	...

than approximately 15 ml. of sulfuric acid be present at the end of the digestion.

Determine and apply a blank correction, which is usually between 0.20 and 0.30 ml.

The results of analyses of eleven organic compounds are indicated in Table I.

DISCUSSION

As shown in Table I, the described method yields results with an average error of −0.04% nitrogen. Even this small difference may be due in part to failure to obtain perfectly pure organic compounds. The results compare very favorably with those of the standard Kjeldahl method with compounds of both high and low nitrogen content.

The disadvantage of the method is the fact that elements such as calcium, barium, copper, and iron interfere by forming precipitates which obscure the end point. Phosphorus also interferes because primary phosphate is titrated to secondary phosphate between the two end points. This renders the procedure inapplicable to fertilizers and many biological samples. The method is applicable to samples containing organic and nitrate nitrogen when the usual sulfuric-salicylic acid modification is applied previous to digestion (*2*, *4*).

With the elimination of the distillation, approximately 20 minutes are saved in each determination.

Work is in progress to adapt the described procedure to the micro scale.

ACKNOWLEDGMENT

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Determination of Carbon and Hydrogen by Combustion

Unitized Dual Apparatus and Improved Procedure

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A dual unitized combustion apparatus and improved procedure are described for the determination of carbon and hydrogen in macrosize samples. The dual feature, absence of rubber connections, provisions for adequate combustion control, and well-defined procedure all combine to give accurate results in the minimum of time. Other noteworthy features are control and indication of all gas flow rates, adequate and convenient pyrometer temperature indication, addition of extra oxygen between the sample and catalyst, use of carefully determined combustion and operating conditions, and a method for accurate analyses of volatile samples. Using the long, precision procedure, a skilled operator can make two determinations in an 8-hour day with a precision of ±0.008% hydrogen and ±0.009% carbon and a probable accuracy of 0.011% hydrogen and 0.015% carbon. Using the short, routine procedure, one experienced operator can make as many as eight determinations daily with a precision of ±0.02% hydrogen and ±0.05% carbon and with a probable accuracy of 0.05% for each element. Both procedures are applicable in presence of sulfur, halogens, nitrogen.

MOST of the progress made in recent years in the determination of carbon and hydrogen has been in the field of microchemistry. Excellent methods and apparatus (*1*, *2*, *5–9*) have been developed which possess the desired attributes of speed, convenience, and accuracy when properly applied, but which too frequently produce results of only moderate accuracy when used on a routine basis (*9*). In this, as in many other laboratories, there has been a definite need for a method which is accurate, precise, and rapid under normal industrial operating conditions. Because macromethods are less susceptible to variations in technique and are thus more suitable for industrial service analysis, it was decided that further development of the macromethod, along lines followed by the microchemists, offered the best possibilities of success.

Wagman and Rossini (*10*) have obtained very precise and accurate results on benzoic acid using large samples (1.8 grams) and improved techniques and apparatus developed at the National Bureau of Standards. In their work on the atomic weight of carbon, Baxter and Hale (*1*) also obtained excellent results using various hydrocarbons and even larger amounts (3 grams) of sample. These investigators had for their prime objective the greatest possible accuracy without regard for time required for analysis. In the present method, accuracy and precision have been achieved by adopting features of the Baxter and Hale apparatus particularly suitable for routine work; speed has been secured by providing a dual apparatus which permits the simultaneous combustion of two samples, by providing convenient and adequate control of the combustion, and by carefully determining the minimum time required for each phase in the analysis.

The conventional combustion method and apparatus for determination of carbon and hydrogen are not generally suitable for industrial use where speed is a consideration, and where analysts may be frequently replaced, and for applications where the greatest precision and accuracy are important. Using the simple apparatus and procedure given in the numerous references on the subject, one usually encounters the following difficulties: (1) loss of much time and effort in establishing the essential conditions, (2) nonsuitability of the apparatus for continuous use, (3) inadequate indications and control of flow rates, (4) inadequate control of furnace temperature, (5) lack of assurance of excess oxygen in combustion tube, (6) errors caused by use of rubber connections, (7) inadequacy of the combustion tube filling for materials containing elements other than carbon, hydrogen, and oxygen, (8) requirement of 4 or more hours per analysis, (9) lack of qualitative evidence of complete oxidation of the carbon and hydrogen, and (10) lack of suitable technique for handling volatile samples. The method described here is a result of a study made to overcome or minimize these difficulties.

The combustion apparatus was unitized in a compact assembly to increase the convenience of use and to allow standardization of technique and procedure; and since it was found that two determinations could easily be made simultaneously, a dual unit

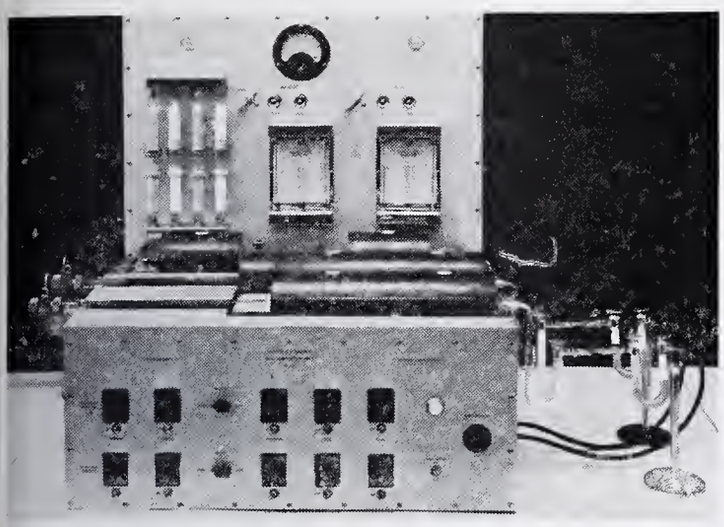

Figure 1. Front View of Dual Combustion Apparatus

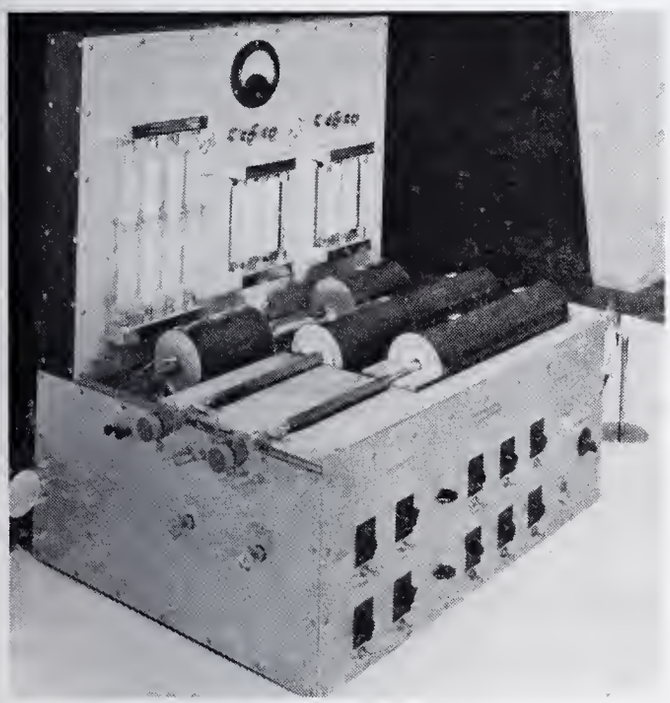

Figure 2. Side View of Dual Combustion Apparatus

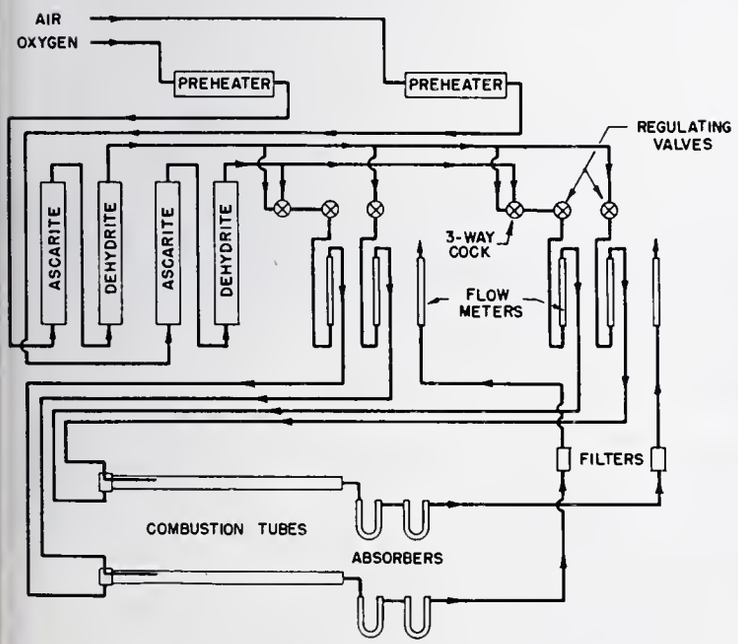

Figure 3. Diagram of Gas Flow through Dual Combustion Unit

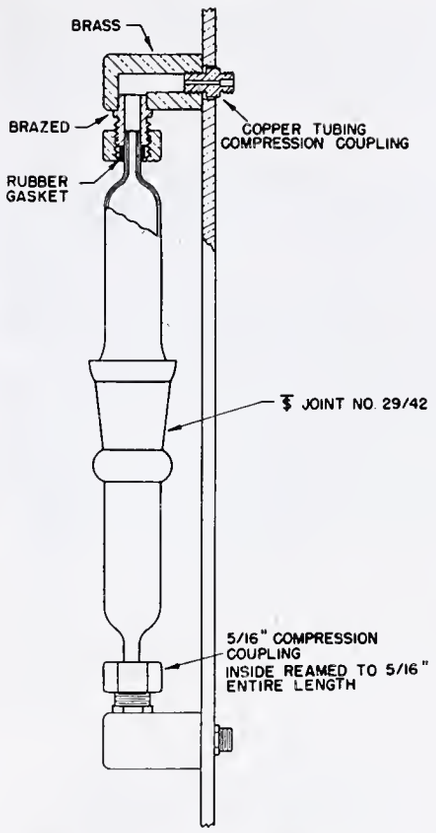

Figure 4. Detail of Purifier Tube and Mounting

was designed to increase the number of analyses per unit time. Proper control and indication of the gas mixture in the combustion tube were achieved by use of microrotameters (0 to 50 ml. per minute) to indicate continuously the rate of flow of air, oxygen, and exit gases and by the use of needle valves to adjust the flow of the air and oxygen. Proper adjustment of furnace temperature was made possible by use of thermocouples and a sensitive direct indicating meter supplied with a suitable selector switch. Excess oxygen in the gases passing over the catalysts was ensured by provision to admit oxygen in the zone between the sample and the catalyst (*3*). Contamination from rubber connections was avoided by use of commercial compression metal fittings, a special metal-to-glass fitting, and standard-taper glass joints. Complete retention of sulfur, halogen, and nitrogen acidic gases was obtained by proper use of lead chromate, silver gauze, and lead peroxide in the combustion tube. Sensitive and immediate control of the rate of volatilization of the sample was obtained by an electric wire heater wound directly on the combustion tube. Errors from contamination of the air and oxygen used were obviated by passing these gases over heated copper oxide in permanently connected heaters.

Permanent connections and mountings were used to allow movement of the apparatus without seriously disrupting its period of usefulness. Special all-glass counterpoises for the absorbers were constructed (having approximately the same net volume, weight, and surface as the actual absorbers) to minimize weighing errors and facilitate correction to a vacuum basis.

APPARATUS

The dual arrangement and general construction of the apparatus are shown in the first two figures. Figure 1 is a front view giving some detail of the absorption train and Figure 2 is a side view showing the gas-inlet and tube-closure assembly arrangement. A diagram of the air and oxygen flow through the apparatus and a schematic arrangement are given in Figure 3. The constructional details of the combustion tube and filling are shown in Figure 6, the electrical wiring diagram in Figure 7, and the arrangement of the absorption train in Figure 8.

The two combustion heaters are mounted one behind the other with their centers about 12.5 cm. (5 inches) apart. Behind and parallel to these are located two preheaters. The panels in front and below the heaters contain control knobs for regulating the temperatures of the various furnaces. The large panel behind and above the furnaces supports the gas purifiers, flowmeters, gas control valves, and pyrometer.

Provision is made for supplying either oxygen or air to the combustion tube. Oxygen is used for combustion of the sample and air for purging out the combustion products, so that the absorbers will be filled with air during the weighing operations.

Both oxygen and air are passed through a preheater furnace and then through a purifying train to remove all traces of water, carbon dioxide, and combustible matter before entering the combustion tube.

Preheater and Purifier. The preheaters are nickel tubes filled with copper oxide and heated to 700° C.

They are similar in construction to the main combustion heaters described below but are not provided with temperature control or with individual switches; therefore, they rapidly reach the operating temperature after the main electrical switch is turned on.

The first purifier tube contains Ascarite and the second tube Dehydrite. A twisted strand of glass wool about the size of a pencil is placed in the lower half of the Ascarite tube to avoid plugging caused by swelling of the Ascarite as it is used. To facilitate cleaning, the purifier tubes have ground joints in the center which are sealed with sealing wax.

The method of mounting the purifier tubes is shown in Figure 4. The mounting consists of $^5/_{16}$-inch compression coupling soldered into brass blocks and reamed out to $^5/_{16}$ inch throughout their length. A short section of medium hard rubber tubing is used as gasket (soft rubber tubing is not satisfactory, as it may wrinkle and cause the joint to leak). The purifier tube is installed in the mount by first placing the nuts and rubber gaskets on the narrow tubes and then pushing one end into the mounting block far enough to allow the other end to enter its mount. This connection is gas tight, yet allows the parts to be easily removed for servicing.

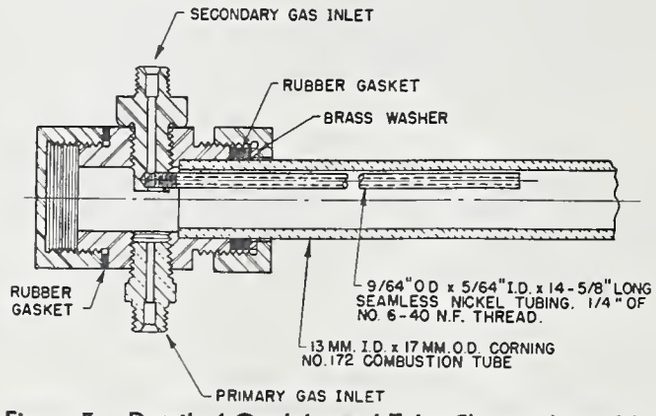

Figure 5. Detail of Gas-Inlet and Tube-Closure Assembly

Combustion Tube. A metal gas-inlet and tube-closure assembly is attached to the front end of each combustion tube; it has a screw cap to provide an opening into the combustion tube and has two gas inlets. The "primary" gas inlet allows gas to enter the front of the combustion tube; the "secondary" inlet conducts the gas, by means of a small-diameter nickel or platinum tube, to a point just in front of the main catalyst section. Figure 5 shows the construction of the gas-inlet and tube-closure assembly and the method of connecting it to the combustion tube. By means of a 3-way cock, either air or oxygen can be passed through the primary inlet, but only oxygen can be passed through the secondary inlet. Each gas line leading to the combustion tube is provided with a packless needle valve for close control of the gas-flow rate and with a rotameter flowmeter. The flowmeters have a useful range of 0 to 60 ml. per minute, and are sensitive to changes of 5 to 10% of the gas flow in the range of 5 to 60 ml. per minute. The mountings for the flowmeters are similar to those for the purifiers.

All the gas lines before the combustion tube are constructed of metal tubing. Rubber is used only for the gaskets on the purifiers, flowmeters, and gas-inlet and tube-closure assembly. The rubber gaskets are always on the outside of the tubes where only a small area of the rubber is exposed to the gas flow; in each case the area is separated from the gas stream by a narrow annular dead air space.

The special combustion tube, shown in Figure 6, is constructed from 13-mm. inside diameter and 17 mm. outside diameter. Corning No. 172 Pyrex combustion tubing. The exit end is fitted with a $^7/_{25}$ standard-taper female joint. A thin-walled capillary tube is sealed through the center of the joint to carry water vapor directly into the absorber without allowing it to come in contact with the connection.

Two types of combustion tube fillings are used: lead peroxide for materials containing nitrogen, and lead chromate for other materials because it is more easily prepared and more desirable from the standpoint of accuracy and ease of operation. Figure 6

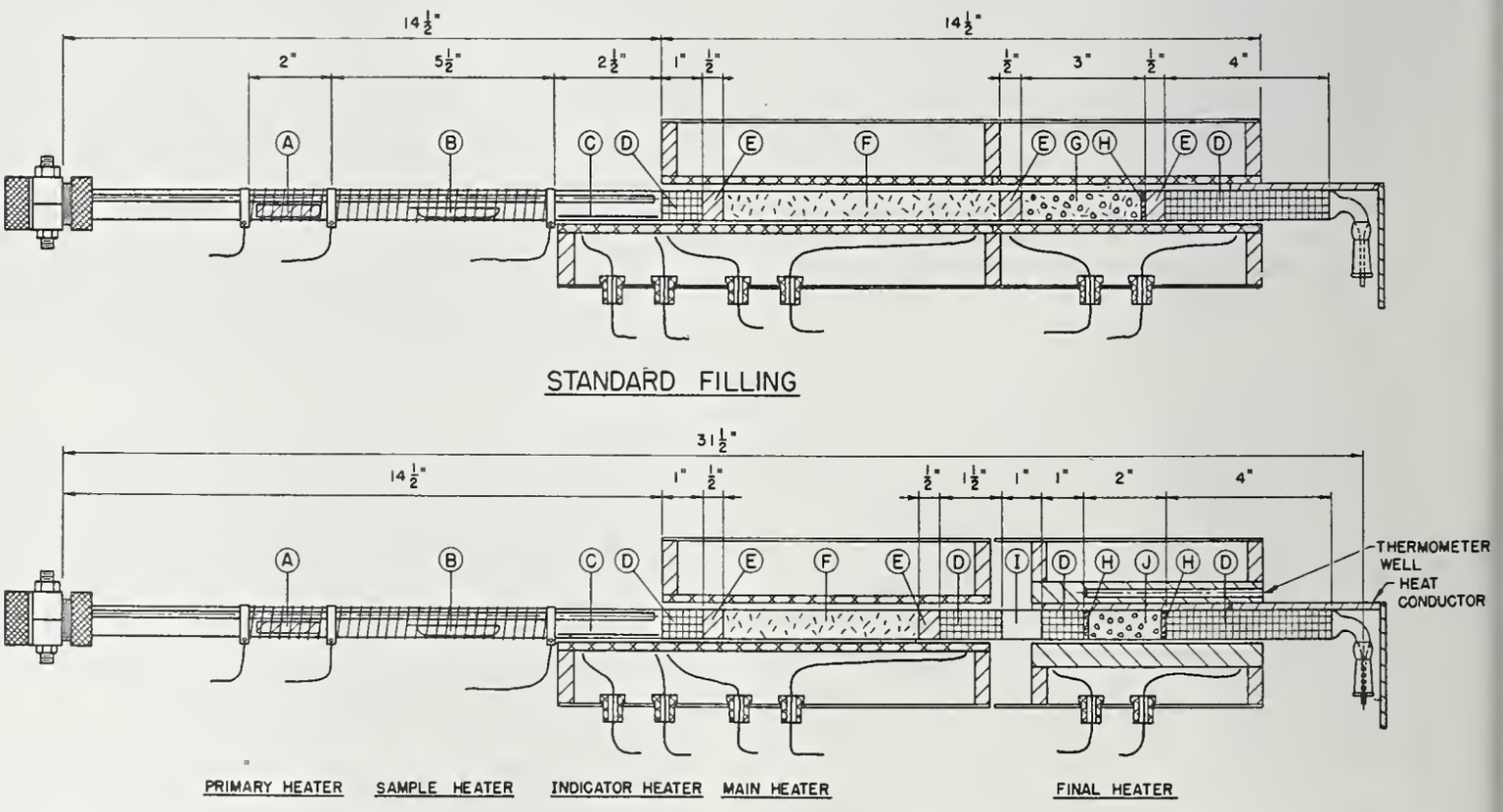

Figure 6. Detail of Construction and Filling of Combustion Tubes

A. 1.5-inch roll of 40-mesh copper gauze
B. Sample Boat
C. Indicator, 2.5 × 0.5 inch rectangular strip of 40-mesh copper gauze
D. Roll of 20-mesh silver gauze
E. Roll of 40-mesh copper gauze
F. 10- to 20-mesh copper oxide wire
G. 50-50 mixture of 10- to 20-mesh copper oxide and lead chromate
H. Ignited Asbestos
I. Empty space
J. 10- to 20-mesh lead peroxide

shows the arrangement of both types of fillings. As indicated, a 1-inch section containing a Pyrex rod separator is provided between the lead peroxide and the copper oxide sections of the filling. This construction reduces the heat transfer between these two sections and allows more accurate control of the temperature of the lead peroxide. In preparing the combustion tube fillings, the copper gauze and the silver gauze are heated in a Bunsen flame before rolling in order to burn off all organic matter and to anneal the gauze, thus simplifying the rolling operation.

Combustion Tube Heaters. The temperature of each combustion tube is regulated by means of five independently controlled heaters. Figure 7 is the wiring diagram of the five heaters and Figure 6 shows their position relative to the combustion tube filling. The "primary" heater and the "sample" heater consist of coils of resistance wire wound directly on the combustion tube as described elsewhere (*4*). The resistance wire is wound on a standard combustion tube and this is then sealed to the exit end from a previously used tube; in this way, the ground joint is used over and over again.

The temperature of the primary heater is controlled by a rheostat placed in series with the heater. The sample-heater temperature is controlled by a variable-voltage autotransformer and two fixed resistances, either of which may be connected in series with the heater by means of a 3-way switch. As shown in Figure 7, these two heaters have a common connection that eliminates a possible cold spot between the two sections. This arrangement requires the use of an autotransformer which has a common lead from input to output. The maximum temperature obtainable with the sample heater is approximately 725° C. A chart is prepared to show the temperatures obtainable with the various combinations of the autotransformer and the 3-way switch settings. Such a chart is very useful in controlling the combustion of the sample.

The indicator heater, main heater, and final heater are all in the main combustion furnace housing. The last two heaters are constructed of resistance wire wound on $1^1/_{16}$-inch inside diameter Alundum tubing. The Alundum tubing is supported in a section of 4-inch steel tubing by means of Transite disks; the space between the Alundum tube and the steel tube is filled with shredded magnesia pipe lagging for insulation. The element for the indicator heater consists of small coils of resistance wire placed in a grooved refractory half-sleeve that is placed under the lower half of the split Alundum tube. The upper half of the Alundum tube is attached to a removable unheated section to permit observation of this part of the combustion tube. The indicator heater element operates from a 50-volt transformer to permit use of heavier wire in the winding.

Two different final heaters are required, the one used in a given combustion depending on the combustion tube filling employed (Figure 6). The heater used with the lead chromate filling is built as an integral part of the standard combustion furnace and is similar to the main heater. The heater used with the lead peroxide filling is constructed as a unit separate from the indicator heater and the main heater, and is constructed of insulated resistance wire wound on a cylindrical block of Duraluminum. Otherwise in construction and insulation this heater is similar to

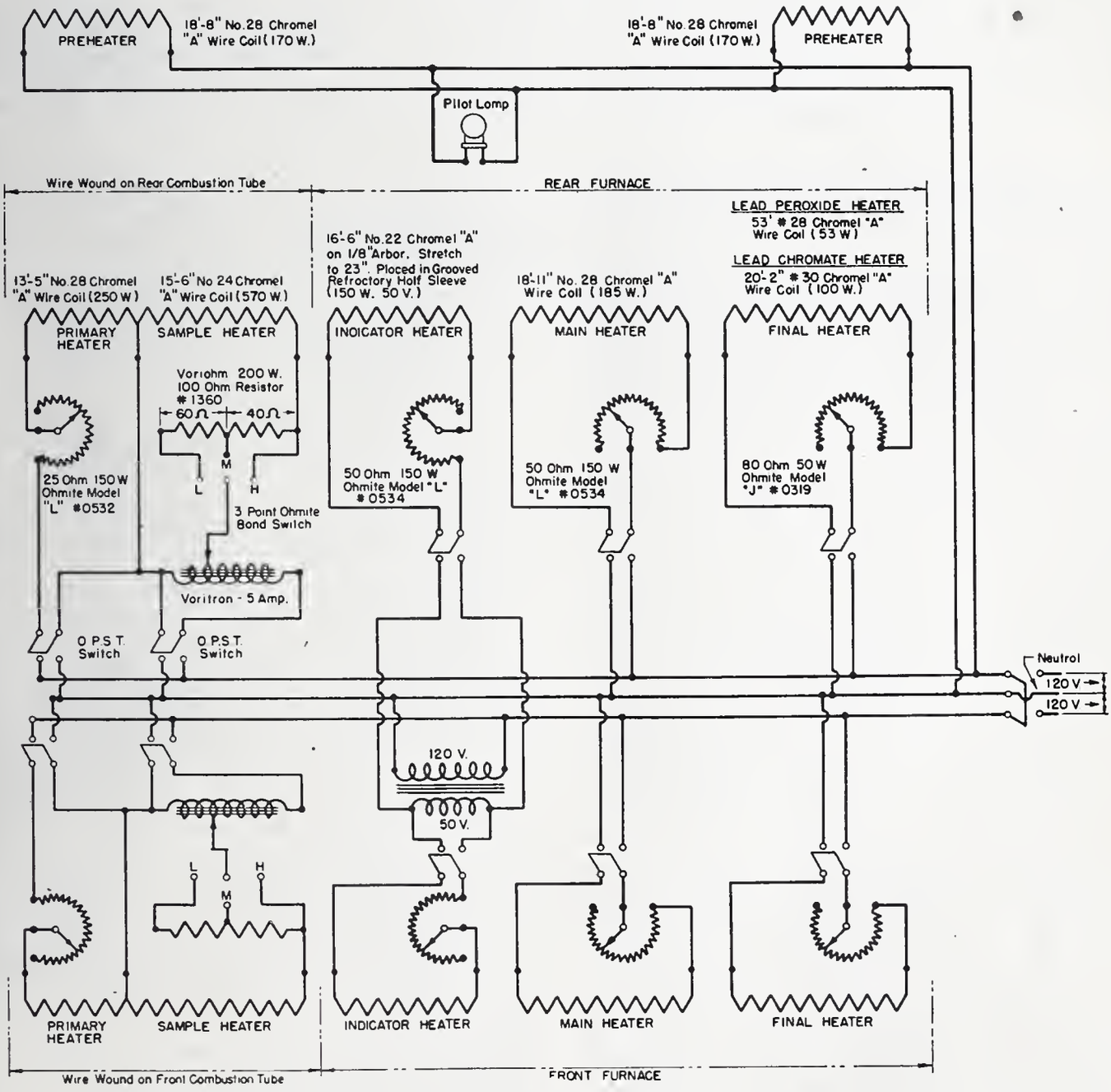

Figure 7. Electrical Wiring Diagram of Dual Combustion Apparatus

the other heaters. The Duraluminum block is fitted with a removable Duraluminum half-sleeve which fits closely around the upper part of the combustion tube as an aid in maintaining close temperature control, yet provides a means of inserting the combustion tube with the resistance winding in place on the front end. The temperature of the three heaters in the main combustion furnace is controlled by means of rheostats connected in series with each heater.

The heaters, as described, are designed to operate on 120 volts. A slight modification of the resistance windings is required when operating at significantly different voltages.

The temperature of the heater for the lead peroxide is determined by means of a short-stemmed thermometer placed in the well in the end of the Duraluminum block. The temperatures of the main heaters, the final heaters for lead chromate, and the preheaters are determined by means of No. 22 gage Chromel-Alumel thermocouples connected through a selector switch to a direct reading meter.

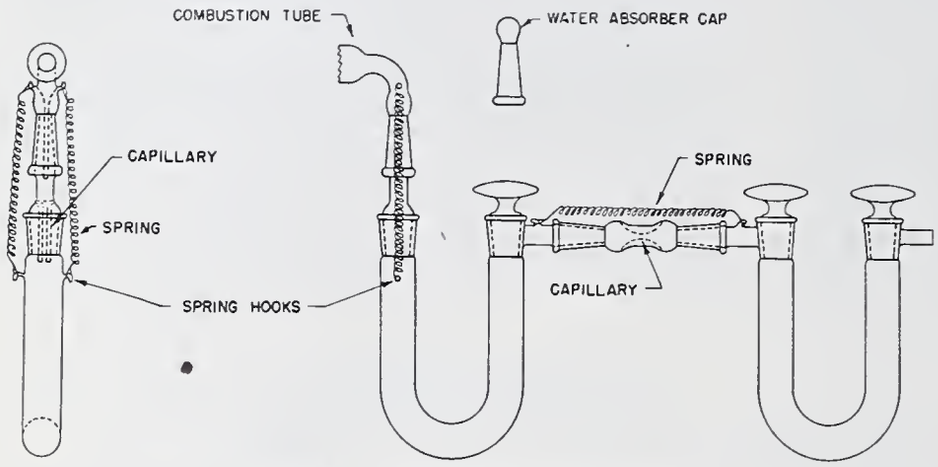

Figure 8. Absorption Train Assembly

A removable Duraluminum heat conductor is placed over the exit end of the combustion tube. It extends a short distance into the final heater and conducts a moderate amount of heat to the end of the combustion tube and to the ground joint, as an aid in preventing condensation of water at these points during the combustion. Holes are drilled through the shield to permit the insertion of thermocouples through it into the combustion furnaces beyond. This shield is shown in position in Figure 6; it is omitted in Figure 1 in order to show more clearly the construction of the end of the combustion tube.

A safety shield (not shown in Figure 1 or 2), constructed with a Pyrex top and a wire screen front and end, is placed over the front exposed part of the combustion tube during the actual combustion of the sample. A compressed air jet is mounted under the sample heater for rapid cooling of this portion of the combustion tube.

Absorption Train. The absorbers consist of specially constructed Pyrex U-tubes as shown in Figure 8. The empty weight of these absorbers is approximately 45 grams. The connection to the combustion tube and between the absorbers is made by means of $^{7}/_{25}$ standard-taper joints held together with springs. To simplify the removal of the lubricant from the joints before weighing, only male joints are sealed to the absorbers. The stopper on the entering side of the water absorber is sealed in place, with a small amount of sealing wax applied to the center of the ground joint, to prevent separation of the joint when the absorber is detached from the combustion tube. A glass cap is provided for the entering side of the water absorber to reduce diffusion of air out of or into the absorber after it has been disconnected from the combustion tube.

The water absorber is filled with Dehydrite; a glass wool plug is placed on top of the filling in the exit side to avoid loss of the finer particles. The carbon dioxide absorber is filled with Ascarite, except for a short section of Dehydrite that is placed on top of the Ascarite in the exit side of the absorber to prevent a possible loss of water vapor. A twisted strand of glass wool is placed along with the Ascarite in the entering side of the absorber to prevent plugging of the absorber by the used Ascarite. Small glass wool plugs are used between the Ascarite and the Dehydrite sections and on top of the filling on both sides of the tube. The stoppers of the absorbers and the various ground connections are lubricated by a soft hydrocarbon grease such as Cello-Grease. The exit side of the carbon dioxide absorbers are connected to the exit-gas flowmeters by means of a short piece of rubber tubing. These flowmeters measure the excess oxygen from the combustion. Glass wool filters are placed immediately before the exit gas flowmeters to remove foreign materials originating from the rubber tubing which might otherwise cause the bobs to stick.

ANALYTICAL PROCEDURE

Two procedures have been developed for use with the described apparatus. The routine procedure is used with samples that require only ordinary precision and accuracy and that must be analyzed in a short period of time. The precision procedure is used for the analysis of samples that require the best possible precision and accuracy. The chief differences in the two procedures are that the precision procedure requires use of a balance sensitive to 0.00001 gram, greater care in preparing and handling the absorbers, and more careful combustion technique along with the use of a larger sample.

Generally, the precision procedure is applied only to the analysis of materials that are mainly made up of carbon, hydrogen, and oxygen and that contain a relatively small proportion of ash, nitrogen, chlorine, sulfur, etc. Best results are obtained with materials, relatively free from nitrogen, which permit the use of lead chromate in the final heater section; however, with proper care and attention to details, satisfactory results can be obtained with nitrogenous materials that require the use of lead peroxide in the final heater section.

Procedure for Routine Analysis. Assemble the combustion unit, fill and install the combustion tubes, fill and attach the absorbers, and adjust the heaters to operate at the following temperatures (° C.):

Primary heater	650 ± 25
Sample heater	700 to 725
Indicator heater	700 ± 25
Main heater	775 ± 25
Final heater	
If lead chromate is used	600 ± 25
If lead peroxide is used	190 to 195
Preheaters	700 ± 50

Purge the combustion train with oxygen and air at a rate of 35 ml. per minute until a 3-hour blank does not exceed 0.3 mg. of water or carbon dioxide; use oxygen during the first 2.75 hours and air for the final 15 minutes of purging time. Turn the primary and sample heaters on only during the first 15 minutes of the purging period.

Prepare and weigh the absorbers in the following manner: Remove the grease from the ground joints with a small cotton swab wet in chloroform. Carefully wipe the absorbers with a clean damp cloth and then wipe over with a clean, dry, lint-free cotton cloth; avoid excessive rubbing with the dry cloth. Place the cap on the entering side of the water absorber and allow the absorbers to stand beside the balance for at least 20 minutes before weighing. (If the relative humidity is below 50%, allow them to stand for a longer period until the static charges have been dissipated.) Weigh the absorbers to ±0.1 mg. by direct comparison with a special tare. Prepare the tare from an empty carbon dioxide absorber by placing in it a quantity of glass that is 2 to 3 grams less in weight and approximately equal in volume to the average of the weight and volume of the filling of the water and carbon dioxide absorber; use a mixture of glass rod and glass tubing sealed off at both ends to secure the required weight and volume of glass. Calculate the volume of the filling from its weight and density; assume that the density of Ascarite is equal to the density of sodium hydroxide. Clean and wipe the tare as described above; keep it in a dustproof (but not air-tight) container near the balance and rewipe the tare only once each week unless laboratory conditions make more frequent attention necessary. Use the same tare for weighing both the water and carbon dioxide absorber. Remove the cap from the water absorber before weighing. Open the stopcock on the exit side of

he carbon dioxide absorber for a few seconds before weighing o equalize the air pressure inside and outside the absorber. eave the stopper on the tare open at all times. Always weigh bsorbers with their gas-space filled with air rather than oxygen.

Take a sample of approximately 0.2 gram, weighed to ±0.1 ıg., for the analysis. Weigh solid or heavy liquid samples in ickel or platinum boats and weigh light liquid samples in sample ubes made from 40-mm. sections of 7-mm. outside diameter 'yrex tubing sealed at one end. Weigh volatile samples in similar ample tubes sealed at one end and drawn out to a fine capillary t the other end. To fill the sample tube, warm the tube in a mall flame and then allow it to cool with the capillary immersed ı the sample. After filling, warm the capillary to expel all the quid from it and then bend the capillary back along side the ample tube, but do not seal the capillary at any point.

Make sure that the primary heater and sample heater are off nd the combustion tube in these sections is cool. Attach the 'eighed absorbers to the combustion tube using a small amount f stopcock lubricant placed at the outer edge of each connection, nd connect the exit side of the carbon dioxide absorber to the xit gas flowmeter. Remove the indicator heater cover, place the afety shield in position, shut off the secondary gas flow, and adıst the primary gas flow to 15 to 30 ml. per minute of air to preent diffusion of room air into the combustion tube. Open the ombustion tube, take out the removable copper spiral and, with ıe aid of a wire hook, insert the sample to a point just beyond ıe center of the sample heater. Place the glass sample tube in nickel boat with the open end or capillary toward the catalyst. teplace the copper spiral in the center of the primary heater and lose the combustion tube. If the sample is extremely volatile, se solid carbon dioxide to cool the sample heater section of the ombustion tube before introducing the sample and to control ıe rate of vaporization of the sample during the combustion.

Adjust the gas flow to give 10 ml. of oxygen per minute in the rimary inlet and 25 ml. of oxygen per minute in the secondary ılet. Use air in the primary inlet only when burning samples 'hich have a tendency to flash or explode. Turn on the primary eater and adjust the temperature to 650° ± 25° C. Turn on he sample heater and gradually increase its temperature until ıe sample begins to vaporize and reduces the indicator strip. The vapors are carried over the copper oxide indicator strip by ıe gas flow from the primary inlet. As soon as the primary gas ow contains an insufficient amount of oxygen for complete oxiation, the front end of the copper oxide indicator strip will beome reduced up to the point where the oxygen coming from ıe secondary inlet builds up an excess of oxygen in the tube. A ıarp line of demarcation is thus established between the reduced ortion and oxidized portion of the indicator strip. The position f this line serves as an immediate guide for controlling the rate f vaporization of the sample, provided a correlation has preiously been made between the position of the line and the mount of excess oxygen in the gas issuing from the combustion ube. Highly volatile samples, and samples that contain sulfur r halogens, often do not appreciably reduce the indicator strip. When the sample is known to contain halogens, the indicator trip should be removed before beginning the combustion to avoid ıe formation of a black deposit on the glass which makes obervation of following determinations difficult.)

Regulate the rate of volatilization of the sample to maintain lways an excess of 10 to 20 ml. of oxygen per minute as indiated by the exit gas flowmeter. Gradually raise the temperaıre of the sample heater to 700° to 725° C.; remove the safety hield and replace the cover over the indicator section to aid in urning out any carbon residue. Readjust the gas flow rates to 0 ml. of oxygen per minute in the primary inlet and 15 ml. of xygen per minute in the secondary inlet. If the capillary sample ube has been used, alternately allow the sample tube to cool and ıen reheat several times to remove any sample vapors and comustion products remaining in the sample tube and to burn any arbon residue.

After all the carbon has been burned, continue the above flow ates for 10 to 12 minutes; then close the secondary gas inlet nd purge for 15 minutes with air admitted through the primary ılet at a rate of 30 to 35 ml. per minute. After starting to purge 'ith air, allow the primary heater and sample heater to cool in reparation for the next determination. Before terminating the urging period, make certain that all water has been purged into he absorber and that no small droplets remain in the vertical ıale joint of the absorber. Remove the absorbers and weigh as efore.

To avoid contamination of tube by materials in the air, always ttach a fresh set of absorbers to the combustion tube before llowing the furnaces to cool. Before beginning an analysis, turn n the furnaces, purge the combustion tube for at least 15 minutes 'ith both oxygen and air after all the furnaces, except the primary heater and the sample heater, have come to operating temperatures.

Procedure for Precision Analysis. Assemble the apparatus and adjust the temperature of the heaters as directed above in the procedure for routine analysis. Purge the combustion train with oxygen and air at a rate of 25 ml. per minute until a 5-hour blank does not exceed 0.3 mg. of water or carbon dioxide; use oxygen during the first 4.5 hours and air for the final 30 minutes of purging time.

Prepare the absorbers as directed under the previous procedure, but allow them to stand 45 minutes or more before weighing them. When weighing the water and carbon dioxide absorbers, use a separate specially prepared tare for each type of absorber. Construct the tares from empty water and carbon dioxide absorbers and prepare them for use in weighing as directed for the preparation of the single tare. Weigh the absorbers by direct comparison with the tare and record the weights to ±0.01 mg. Just before weighing the carbon dioxide absorber, open one of the stopcocks on the absorber and on the tare for a few seconds, but have the stopcocks closed during the weighing operation. Weigh the water absorber with the cap removed from it and its tare, but replace the caps as soon as possible.

Take a 0.5-gram sample for the analysis; weigh the sample, contained in an appropriate container, to 0.01 mg. Connect the absorbers, insert the sample, and make the combustion exactly as directed in the procedure for routine analysis, but use an oxygen flow rate of only 5 ml. per minute in the primary inlet and 15 ml. per minute in the secondary. Burn the sample at such a rate as to maintain the excess oxygen flow rate at 5 to 10 ml. per minute until combustion is essentially complete. Readjust the oxygen flow rates to 10 ml. per minute in each inlet while burning any carbon residue, and continue for 10 to 15 minutes after all the carbon has been burned; then purge with air through the primary inlet at a rate of 20 to 25 ml. per minute for 30 minutes. Remove the absorbers and weigh as before. Use fresh carbon dioxide absorbers for each analysis and renew the water absorber before it is one half used up.

Calculation of Results. The possible accuracy of both the routine and precision procedures is good enough to justify applying vacuum corrections to the weights of the sample, water, and carbon dioxide. When volatile samples are being analyzed, the correction to the sample weight must include the weight of air displaced by both the liquid and the vapor (errors introduced by neglecting the correction for the vapor may be as great as 0.1%). Based on the calculations by Baxter and Hale (*1*), the volume change of Ascarite on absorbing carbon dioxide is taken at 0.44 ml. per gram of carbon dioxide absorbed. On the basis of similar calculations, the volume change of Dehydrite on absorbing water is taken at 0.76 ml. per gram of water absorbed. This value is based on the assumption that only magnesium perchlorate hexahydrate is formed by the reaction of magnesium perchlorate and water. Although some trihydrate is also formed, the above assumption is probably sufficiently correct for the purpose of making vacuum corrections. In calculating the results reported in this paper, the atomic weights of hydrogen and carbon were assumed to be 1.0080 and 12.010, respectively.

EXPERIMENTAL

A considerable number of experimental determinations and variations were made during the development of the apparatus and the procedures. Throughout this experimental work, the precision procedure was used, so that the results could be applied to both procedures whenever possible. The samples and absorbers for the precision method were weighed by the method of swings on a Troemner balance with a sensitivity of 0.3 mg. per scale division. Weighings for the routine method were made on a Becker, chain-o-matic, magnetic-damped balance with a sensitivity of 0.3 mg. per scale division.

Time Required for Equilibration of Absorbers before Weighing. The two main factors which govern the time required for the wiped absorbers to reach a constant weight are: (1) equilibration of the outside surface of the glass by dissipation of static charges placed on the glass during the wiping operation and by equilibration of the glass surface and the moist air;

Table I. Precision of Weighing of Absorbers

Expt. No.	Date	No. 1 Dehydrite	No. 1 Ascarite	No. 2 Dehydrite	No. 2 Ascarite
1	1–4	1.10820	3.38472	4.97148	4.56806
2	1–4	1.10809	3.38460	4.97115	4.56798
3	1–4	1.10813	3.38468	4.97121	4.56805
4	1–5	1.10830	3.38468	4.97129	4.56802
5	1–5	1.10826	3.38469	4.97144	4.56799
6	1–5	1.10832	3.38467	4.97144	4.56806
7	1–5	1.10833	3.38461	4.97136	4.56793
8	1–8	1.10854	3.38469	4.97170	4.56792
9	1–8	1.10859	3.38461	4.97177	4.56797
10	1–8	1.10861	3.38465	4.97167	4.56790
11	1–8	1.10889	3.38463	4.97178	4.56789

Table II. Time Required for Removal of Combustion Products during Purging

		Gain in Weight of Absorbers after Purging with Air at a Rate of 20 Ml. per Minute									
		Up to 10 Minutes		10 to 30 Minutes		30 to 60 Minutes		60 to 180 Minutes		Up to 300 Minutes	
Detn. No.	Benzoic Acid	H_2O	CO_2	H_2O	CO_2	H_2O	CO_2	H_2O	CO_2	H_2O	CO_2
	Gram	*Gram*	*Gram*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
1	0.2345	0.1045	0.5929	0.11	0.00	0.13	−0.04	0.13	−0.03	...	...
2	0.2272	0.0999	0.5689	0.10	0.04	−0.02	0.01	0.01	0.01	...	...
3	0.2561	0.1144	0.6478	0.09	0.06	0.16	0.07	0.26	0.10	...	...
4	0.2470	0.1108	0.6224	0.14	0.11	0.12	0.06	0.13	0.04	...	...
5	0.00	...	...	...	...	...	...	...	...	0.28	0.17
6	0.00	...	...	...	...	...	...	...	...	0.39	0.24

(2) equilibration of the gas inside the absorber to room temperature and pressure. The effects of these two factors on the final weight of the absorber were determined independently.

The time required for the equilibration of the outside of the absorber was studied by observing the rate of change of weight of an empty carbon dioxide absorber after it had been wiped by several different methods; the absorber was weighed by direct comparison with a tare that consisted of a similar empty carbon dioxide absorber that had been wiped several hours before it was used. Both the absorber and the tare were weighed with the stoppers closed in order to eliminate any effect of temperature on the gas density inside the absorber. A small amount of radium bromide was present in the balance case during the experiment as a possible aid in dispelling static charges. The tests were made at 23° to 27° C. and at a relative humidity of approximately 70%.

The rate of equilibration of the air temperature and resulting gas density inside the absorber was determined by heating or cooling an empty carbon dioxide absorber to a temperature 10° to 15° C. different from the room temperature and observing the rate of change of weight by weighing as before. The stopcocks on both the absorber and the tare were left open during the experiments. The absorber was wiped at least one hour before beginning the test, so that the static charges would have been dissipated.

The details of the various experiments are given in the following list and the results are plotted in Figure 9.

Experiments. 1. The absorber was first wiped with a damp cotton cloth and then with an air-dry cotton cloth. Rubbing with the dry cloth was kept at a minimum. After the absorber had been wiped, the fingers were passed lightly over the surface of the glass as a possible aid in removing static charges.

2. The conditions were the same as those in 1 except that the absorber was rubbed for approximately 2 minutes with the dry cloth.

3. The conditions were the same as those in 2 except that the absorber was not touched with the fingers after wiping.

4. Experiment 3 was repeated except that an oven-dried cotton cloth was substituted for the air-dry cloth. This cloth left a great deal of lint on the absorber.

5. The conditions were the same as those in 3 except that a dry wool flannel cloth was used in place of the dry cotton cloth.

6. The absorber was cooled to approximately 10° C. in a refrigerator.

7. The absorber was heated in the oven to approximately 35° C.

These experiments indicate that with proper wiping and with the absorbers at room temperature, the weight of the absorber will be within 0.1 mg. of the final weight after standing 20 minutes and the difference will be less than 0.05 mg. after standing 45 minutes. In localities where the relative humidity is much lower, longer time of standing will probably be required for proper equilibration.

Precision of Weighing of Absorbers. By repeated wiping and weighing of four typical absorbers, it was shown that these operations can generally be carried out with a precision of 0.05 to 0.10 mg.; in a few cases deviations as great as 0.3 mg. are encountered. A summation of these trials is given in Table I.

Purging the Combustion Train. The time required for purging the combustion train was determined, using samples of benzoic acid, by changing and weighing the absorbers at appropriate intervals after the combustion had been judged complete as shown by absence of any residue in the sample heater section. The results, given in Table II, show that only negligible quantities of water and carbon dioxide remain after purging for 10 minutes at a rate of 20 ml. per minute after the combustion is complete. A longer purging time has been specified in both procedures in order to provide a desirable safety factor and to allow sufficient time for the oxygen in the combustion train and the absorbers

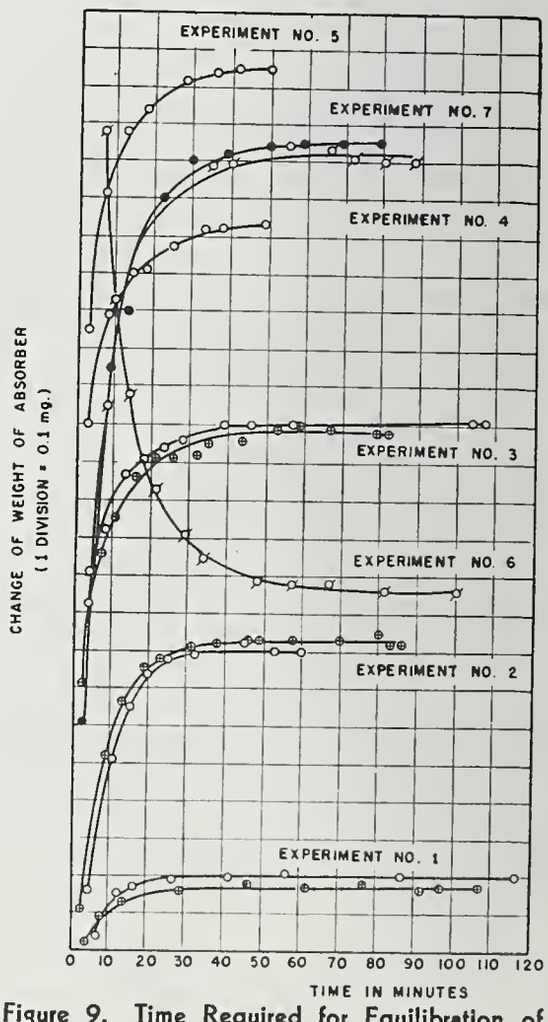

Figure 9. Time Required for Equilibration of Absorber

Table III. Effect of Oxygen Flow Rate and Copper Oxide Catalyst Temperature upon Completeness of Combustion

Material	Detn. No.	Sample	Oxygen Flow Rate Primary	Secondary	Total	Excess Flow Rate Average	Minimum	Time for Combustion	Catalyst Temp.	Hydrogen	Carbon
		Gram	*Ml./min.*	*Ml./min.*	*Ml./min.*	*Ml./min.*	*Ml./min.*	*Hours*	° C.	%	%
Neutral oil	1	0.51710	5	10	15	8	2	2	710	13.658	86.324
	2	0.55591	5	10	15	6	2	3	750	13.657	86.300
	3	0.62633	5	10	15	5	1	3	750	13.654	86.278
	4	0.60760	10	35	45	25	8	1.5	675	13.463	85.497
	5	0.57113	10	35	45	27	20	1.25	700	13.576	85.900
	6	0.55941	10	35	45	25	18	1.25	700	13.469	85.335
	7	0.60889	10	35	45	25	7	1.25	725	13.638	86.242
	8	0.52830	10	35	45	25	17	1.25	725.	13.634	86.206
	9	0.58596	5	40	45	23	15	1	750	13.660	86.279
	10	0.59493	15	30	45	20	13	1.25	750	13.660	86.299
p-Phenyl phenol	11	0.56564	15	30	45	22	18	1	750	5.946	84.723
	12	0.55395	5	10	15	5	2	2	750	5.929	84.732
								Min.			
Anthracene	13	0.16620	5	10	15	6	2	40	750	5.705	94.221
	14	0.17016	15	30	45	20	19	15	750	5.705	94.224
Chrysene	15	0.20829	5	10	15	7	1	55	750	5.468	94.090
	16	0.26275	15	30	45	20	13	25	750	5.458	94.074

Table IV. Reproducibility of Routine and Precision Procedures

Detn. No.	Precision Procedure Neutral oil	Hydrogen	Carbon	Routine Procedure Neutral oil	Hydrogen	Carbon
	Gram	*% by wt.*	*% by wt.*	*Gram*	*% by wt.*	*% by wt*
1	0.54308	13.653	86.308	0.2057	13.66	86.42
2	0.51775	13.670	86.322	0.2239	13.75	86.18
3	0.61875	13.657	86.306	0.2050	13.67	86.36
4	0.63619	13.667	86.173	0.1757	13.70	86.28
5	0.55575	13.650	86.311	0.1918	13.65	86.34
6	0.51726	13.640	86.304	0.2033	13.66	86.37
7	0.57089	13.652	86.317	0.2050	13.68	86.41
8	0.56751	13.642	86.304	0.2045	13.66	86.41
9	0.49913	13.654	86.312	0.1969	13.66	86.35
10	0.45472	13.647	86.331	0.2089	13.68	86.36
11	0.40530	13.656	86.327	0.2100	13.64	86.35
12	0.45003	13.646	86.329	0.2083	13.66	86.31
13	0.51944	13.656	86.319			
Average		13.652[a]	86.316[a]		13.67[b]	86.36[b]
Standard deviation		±0.008[a]	±0.009[a]		±0.02[b]	±0.05[b]

[a] Does not include No. 4. [b] Does not include No. 2.

The following blanks were taken before and after the above determinations; a correction was not applied to the results.

Time, Hours		Front Tube, Mg. H_2O	CO_2	Rear Tube, Mg. H_2O	CO_2
Before	3.5	0.09	0.00	0.07	0.02
After	4.5	0.11	0.07	0.35	0.07

o be displaced completely by air. In the routine procedure, he period of purging may be used advantageously for weighing a ew set of absorbers and samples.

Effect of Catalyst Temperature and Oxygen Flow Rate pon Completeness of Combustion. Several different types f compounds were burned at various copper oxide catalyst tem- eratures and at different oxygen flow rates; the results of these ests are given in Table III. The samples used were commercially vailable materials of unknown purity. For the purpose of these omparisons, it was assumed that the combustion was complete vhen duplicate results were obtained at two widely different xygen flow rates. The results obtained indicate that the copper xide catalyst must be maintained above 750° C. unless low xygen flow rates and, consequently, longer combustion times re used. However, temperatures above 800° C. must be avoided, ince Corning No. 172 Pyrex generally is not satisfactory at such emperatures, even when well supported.

Precision and Accuracy. The precision of both procedures vas determined by repeated analysis of a sample of neutral oil; he results are given in Table IV. The probable deviation is ±0.02% hydrogen and ±0.05% carbon for the routine pro- edure, and is ±0.008% hydrogen and ±0.009% carbon for the recision procedure. It is believed that the precision of the nethod is determined to a great extent by the accuracy of the veighings. The precision of the precision method could probably be further improved by the use of larger samples. The accuracy of the routine method has been determined by analysis of several Bureau of Standard samples issued as standards for the microdetermination of carbon and hydrogen and of several compounds of better than ordinary purity. The results of these tests (Table V) indicate that the accuracy is in the order of 0.05% for both elements. The naphthalene, toluene, nitrobenzene, and *p*-nitrophenol were purified by several sublimations, crystallizations, or fractional distillations, as the case may be, of ordinary C.P. or high purity chemicals. The benzoic acid and ammonium thiocyanate were Mallinckrodt's analytical quality. The Bureau of Standards sample of benzoic acid was also analyzed according to the precision procedure. The results, given in Table VI, show an accuracy of 0.011% for hydrogen and 0.015% for carbon.

Time Required for Analysis. Table VII gives the average time required for the various operations necessary for two simultaneous determinations by the routine procedure and the precision procedure. The over-all working time can be slightly decreased from the value given by using the weighed absorbers from one analysis for alternate subsequent determinations. Approximately 5 years of experience indicate that six to eight determinations can be made in an 8-hour day by the routine procedure, provided the furnaces are at the operating temperature at the beginning of the day.

DISCUSSION

The merits of the described apparatus and procedures are principally due to the adaptation of previously known practices and to the emphasis placed on adequate control of the combustion operations rather than to the development of new ideas and techniques. The principle of introducing oxygen at two points in the combustion tube was successfully used by Dennstedt (*3*), but this idea has not usually been incorporated in the classical methods. In the present apparatus, the double oxygen inlet is advantageously used in combination with the indicator strip and the sensitive flowmeters for instant indication of the entering and excess oxygen flow rates. This system provides a convenient and safe means of controlling the combustion so that the combustion can be made at the maximum possible rate while still maintaining the proper conditions to ensure complete oxidation. With the catalyst temperature and flow rates employed, experience has shown that excess oxygen must be present at all times in the catalyst section in order to ensure complete combustion of materials that crack easily to form methane, etc. There is also some indication that the use of the double oxygen inlet materially reduces the possibility of explosion when burning inflammable materials.

The rotameter-type flowmeter is a decided improvement over the conventional bubble-counter-type flowmeter. The rotameter flowmeter acts instantly, continuously indicates the prevailing rate of flow, and becomes more sensitive as the flow rate is decreased. The gas flow rates employed in the authors' procedures are higher than rates at which bubbles can be counted and thus

Table V. Accuracy of Routine Procedure

Material		Carbon[a]	Hydrogen[a]
		% by weight	
Naphthalene		93.75	6.40
		93.83	6.35
		93.64	6.30
		93.66	6.30
	Average	93.72	6.34
	Theory	93.71	6.29
Toluene		91.05	8.84
		91.12	8.85
		91.15	8.82
		91.18	8.84
	Average	91.12	8.84
	Theory	91.25	8.75
Benzoic acid (Mallinckrodt's analytical reagent quality)		68.84	5.08
		68.90	4.99
		68.87	4.98
		68.84	4.98
	Average	68.86	5.01
	Theory	68.84	4.95
Nitrobenzene		58.58	4.08
		58.53	4.08
		58.53	4.07
	Average	58.55	4.08
	Theory	58.53	4.09
p-Nitrophenol		51.89	3.61
		51.88	3.60
		51.78	3.61
	Average	51.85	3.61
	Theory	51.80	3.62
Ammonium thiocyanate		15.95	5.32
		15.99	5.37
	Average	15.97	5.34
	Theory	15.78	5.30
Acetanilide (NBS standard sample 141)		71.13	6.80
		71.10	6.72
		71.06	6.77
	Average	71.10	6.76
	Theory	71.09	6.71
Cystine (NBS standard sample 143)		29.89	5.10
		29.95	5.08
		29.95	5.09
	Average	29.93	5.09
	Theory	29.99	5.04
Benzoic acid (NBS sample 39f)		68.79	5.01
		68.78	5.02
		68.76	5.00
	Average	68.78	5.01
	Theory	68.84	4.95

[a] Corrected to a vacuum basis.

Table VI. Accuracy of Precision Procedure

Material		Carbon[a]	Hydrogen[a]
		% by weight	
Benzoic acid (NBS standard sample 39f)		68.830	4.963
		68.830	4.966
		68.828	4.962
	Average	68.829	4.964
	Theory	68.843	4.953

[a] Corrected to a vacuum basis.

Table VII. Time Required for Two Simultaneous Analyses by Routine and Precision Procedures

Operation	Time in Minutes: Routine procedure	Precision procedure
Weigh absorbers (4)	8	45
Weigh samples (2)	8	35
Connect absorbers, insert samples	7	7
Make combustion	55	155
Purge with air	15	30
Prepare absorbers for weighing	7	7
Equilibrate absorbers	20	45
Weigh absorbers (4)	8	45
Calculate results	3	10
Total elapsed time for 2 analyses	131	379
Total working time for 2 analyses	96	304

give a reliable indication of the flow rate. Furthermore, when bubble counters are used, there is always a possibility of contaminating the gases or connecting lines with the liquid used in the counter.

The use of resistance wire wound directly on the combustion tube to heat the sample section was used successfully by Baxter and Hale (*1*). The low heat capacity of this type of sample heater makes possible a rapid change in heating rate by the simple process of adjusting a rheostat or a variable transformer. It provides complete visibility of the sample during combustion, adequate control at all times, and quick cooling after the combustion is complete. An attempt was made to control the combustion of the sample by the use of a fully heated furnace section which was slowly moved forward over the sample by means of a crank and screw arrangement similar to that used for microcombustions (*5*, *8*) except that it was operated manually. This arrangement was moderately successful, but it required considerably more attention from the operator than the resistance wire wound directly on the glass tube. When the described heater is used, the combustion proceeds with very little attention once the proper rate has been established.

The ground joint with sealed-in capillary used on the end of the combustion tube is similar to that employed by Baxter and Hale (*1*). This design, together with the Duraluminum heat transfer block, has eliminated any trouble arising from the condensation of water in the exit end of the combustion tube.

During 5 years, approximately 6500 carbon and hydrogen determinations have been made with the aid of one dual unit. During this time, the apparatus and procedure have given satisfactory results in the routine analysis of the following materials:

(1) Hydrocarbons, such as paraffins, aromatics, naphthenes, aviation gasoline and other low-boiling materials.
(2) Oxygen-containing materials, such as alcohols, phenols, glycols, ethers, esters, ketones, aldehydes, and acids.
(3) Sulfur-containing materials, such as mercaptans, sulfides, disulfides, and thiophenes.
(4) Nitrogen-containing materials, such as amines, amides, cyanides, cyanates, hydrazines, pyridines, and nitro compounds.
(5) Halogen-containing materials, such as alkyl mono- and dichlorides, and alkyl bromides.
(6) Various mixtures of the above-mentioned elements.
(7) Esters of iron, aluminum, and copper.

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Microdetermination of Halogens and Sulfur

Using the Grote Combustion Apparatus

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Automatic combustion methods for the determination of sulfur, chlorine, bromine, and iodine are described. By the use of a modified Grote combustion tube in combination with volumetric procedures, satisfactory micromethods have been developed. The indicators used are dipotassium rhodizonate for sulfur, dichlorofluorescein for chlorine and bromine, and starch solution for iodine. The largest standard deviation for these methods was found to be ±0.14% for sulfur, ±0.10% for chlorine, ±0.17% for bromine, and ±0.15% for iodine in the group of compounds analyzed. Methods are also described for the acid-base titration of sulfur (in the absence of acid-forming elements other than carbon) and for the determination of traces of chlorine employing a turbidimetric estimation of the chloride ion.

COMBUSTION methods for the determination of halogens and sulfur were among the first microchemical procedures suggested by Pregl. Other methods for the decomposition of the sample, such as the micro-Carius and the Parr microbomb, have been described by Niederl and Niederl (*9*). Huffman (*7*) has described a combustion method for sulfur in which the sulfur oxides are adsorbed on metallic silver. Hallett and Kuipers (*6*) have described a combustion and titration procedure for sulfur similar to that used in this laboratory and have summarized the literature on the sulfur determination. Another modified combustion method originally developed on the macro scale for halogens and sulfur by Grote and Krekeler (*4*) and later on a micro scale for sulfur by Schöberl (*13*) has been described and used in Germany. (Reference is made in this paper only to Grote, in order to conform with the original Jena literature describing the apparatus.) Wurzschmitt and Zimmerman (*15*) modified the original Grote combustion tube and used it in the macrodetermination of halogens, sulfur, mercury, and selenium in organic compounds; sulfur in volatile inorganic materials such as iron pyrites; and carbon by the absorption of the carbon dioxide in alkali followed by a modified Winkler titration technique.

Previous to the war, a Grote microapparatus was obtained from Germany and was used in this laboratory for a number of years. During the past few years, Grote combustion tubes made from fused quartz and also from Vycor silica glass No. 790 have been used. The apparatus is now available from domestic sources (Arthur H. Thomas, Philadelphia, Pa.; Corning Glass Works, Corning, New York; Thermal Syndicate, Ltd., New York, N. Y.).

COMBUSTION FURNACE

Electrical units have been found to be the most satisfactory means for heating the combustion tube. Figure 1 shows an automatic apparatus as set up in this laboratory. It is similar in construction to that previously described (*11*) for the determination of nitrogen by combustion, but other designs, such as those of Hallett (*5*), Clarke and Stillson (*2*), and another design described by the authors (*12*) would also be suitable.

Three furnaces are used, as can be seen in Figure 1. One is a short movable furnace (8.5 cm. long) heated to 750° C., which is used for the combustion of the sample. The speed and temperature of the movable furnace may be varied to suit the rate of combustion of the material being burned. This is followed by a long stationary furnace (18.5 cm. long) also at 750° C., which heats the portion of the combustion tube containing the sintered frits. The function of the third short furnace (8.5 cm. long), which is heated to 350° C., is to prevent condensation of vapor in the exit end of the combustion tube and in the ground-glass joint which connects the two pieces of apparatus. Details on construction have been amply described in the references given.

COMBUSTION TRAIN AND AUXILIARY EQUIPMENT

The combustion train consists of a Grote combustion tube and a Grote absorber through which oxygen is drawn by means of a slight vacuum. An aspirator pump or house vacuum is satisfactory. Before entering the combustion tube, the oxygen passes through a pressure regulator filled with concentrated sulfuric acid, a safety trap, and a U-tube containing Ascarite to remove any sulfuric acid mist. Figure 2 shows the Grote combustion tube with specifications, and Figure 3 the Grote absorber. Figure 1 shows them assembled in the authors' setup.

The combustion tube may be made of fused quartz containing sintered-quartz frits or of Vycor silica glass No. 790, in which case the frits are of the corresponding material. Glass is not satisfactory because of the high temperatures required in the sintered-frit portions of the combustion tube. A satisfactory porosity of the frit in the absorber is such that 0.5 ml. of water per minute will flow through the frit at atmospheric pressure. Similar measurements of the two frits in the combustion tube gave a flow rate of 1.33 ml. of water per minute. It is felt that the porosity of the frits in the combustion tube should not be greater than that indicated above.

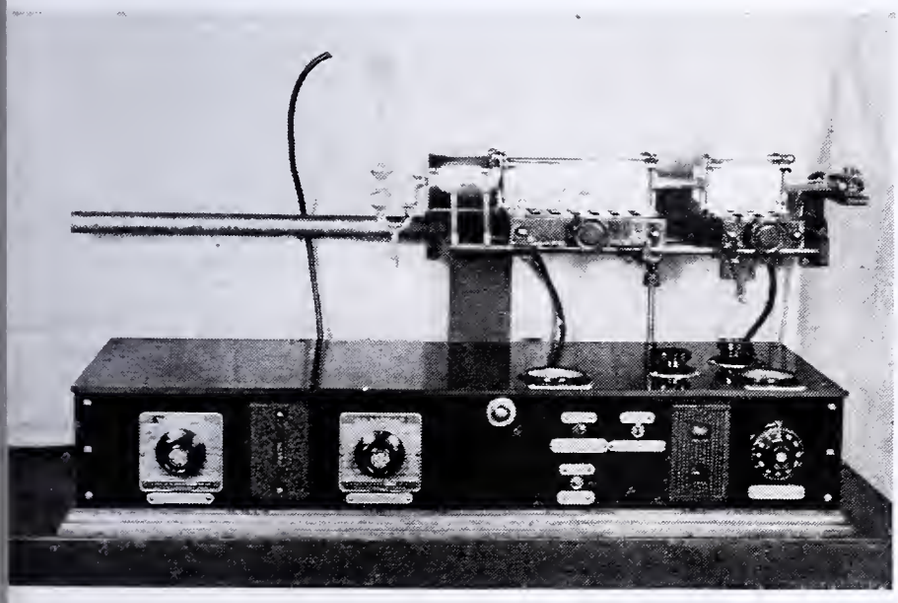

Figure 1. Automatic Apparatus Setup

REAGENTS

Combustion is carried out in an atmosphere of oxygen. The oxygen need not necessarily be free of water, but must not contain halogen or sulfur. The following reagents are required:

FOR DETERMINATION OF SULFUR. Barium chloride, 0.01 *N* (standardized gravimetrically by barium sulfate method).

Bromine, saturated water solution.

Sodium hydroxide, 0.1 *N*.

Hydrochloric acid, 0.05 *N*.

Phenolphthalein indicator solution.
Alcohol, 95% (3A grade).
Sulfuric acid, 0.01 *N* (standardized by acid-base titration against sodium hydroxide which has been standardized against Bureau of Standards potassium acid phthalate).
Dipotassium rhodizonate indicator, made by dry grinding 0.5 gram of dipotassium rhodizonate with 49.5 grams of cane sugar.
For Determination of Chlorine and Bromine. Hydrogen peroxide (reagent grade), 3%, made by diluting 30% Superoxol.
Silver nitrate, 0.01 *N*.
Sodium acetate, saturated water solution.
Acetone, C.P.
Dichlorofluorescein indicator prepared by dissolving 0.01 gram of dichlorofluorescein in 100 ml. of 60% alcohol to which are added 2.5 ml. of 0.01 *N* sodium hydroxide.
Hydrazine sulfate, saturated water solution (bromine determination only).
For Determination of Iodine. Sodium hydroxide, 5%.
Sodium acetate, 20%.
Solution of sodium acetate in glacial acetic acid, 10%.
Bromine, free from iodine.
Formic acid, 90%.
Potassium iodide.
Sodium thiosulfate, 0.01 *N*.
Sulfuric acid, 1 to 2.
Starch indicator solution.
Potassium biiodate for standardization of the sodium thiosulfate solution.

PROCEDURE FOR COMBUSTION

The absorbing solution used in the Grote absorber depends on the nature of the analysis. Sodium hydroxide solutions, 0.1 *N* and 5%, are used in the determination of sulfur and iodine, respectively, while 3% hydrogen peroxide is used in the determination of chlorine and bromine. Dilute hydrogen peroxide cannot be used as the absorbing medium for sulfur determinations where dipotassium rhodizonate is used. Apparently even traces of hydrogen peroxide remaining in solution after attempts to destroy it by boiling in acid solution tend to destroy the color of this indicator. This has also been noted by Hallett and Kuipers (*6*).

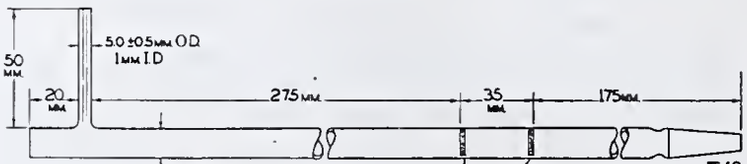

Figure 2. Grote Combustion Tube

Five milliliters of the appropriate solution are introduced into the top of the absorber. By means of a rubber pressure bulb (5 × 7 cm.), approximately one half of the solution is forced through the frit into the U-portion of the absorber while the other half remains above the frit. The absorber, previously heated over a flame to remove moisture from the joint, is connected to the ground joint of the combustion tube in the 350° C. furnace. Since the thermal coefficients of expansion of the combustion tube and the absorber are different, the joint must be disconnected while the apparatus is still hot. The exit end of the absorber is connected to the vacuum line, the vapor trap being inserted between the absorber and the source of vacuum. It has been found that the rate of oxygen flow through the tube can vary between wide limits. In general, a fairly rapid stream of oxygen, about 60 ml. per minute, should be maintained. This rate of flow corresponds to about 70 bubbles of oxygen per minute passing through the U-portion of the Grote absorber which serves as flowmeter or bubble counter.

The platinum boat containing the weighed sample which may vary from 4 to 20 mg., depending upon the per cent halogen or sulfur in the compound, is inserted into the combustion tube at a distance of about 3 cm. from the stationary furnace. The combustion tube is then closed with a rubber stopper, the movable furnace brought to within about 5 cm. of the stationary furnace, and the automatic time switch set for a 20-minute period of combustion. In the authors' apparatus, this switch starts the motion of the movable furnace and also turns on the current to heat the furnace to the required temperature of about 750° C. During this 20 minutes the furnace will have moved over the sample and the combustion will be complete.

Some difficulty has been experienced with back-firing. The original Grote combustion tube has a one-hole disk fused inside the tube about 5 cm. in front of the first sintered frit. As originally designed, the function of this disk was to prevent back-firing during the combustion. On the basis of a large number of determinations, it was felt that this disk was not desirable. It was thought that the tendency of low-melting compounds to back-fire was apparently due to the abnormal concentration of vapors and liquid distillate collecting in front of this disk, thus causing the formation of explosive mixtures. A modified Grote combustion tube was constructed without this disk. In the use of this tube, a roll of platinum gauze about 6 cm. long is inserted in front of the first frit so that about 2 cm. extends out of the stationary furnace. Using this procedure, the back-firing has been greatly reduced.

Table I. Determination of Efficiency of Grote Absorber

Sulfur Taken, Mg.	0.01 *N* NaOH Required, Ml.	Sulfur Found, %
5.032	31.45	100.2
4.138	25.88	100.3

DETERMINATION OF SULFUR

Compounds Containing Only Carbon, Hydrogen, Oxygen, and Sulfur. It is obvious that if the compound contains only carbon, hydrogen, oxygen, and sulfur, the sulfur determination can be carried out as an acid-base titration. In this case, the oxidation products are absorbed in neutralized 3% hydrogen peroxide solution, and after any carbon dioxide has been expelled by boiling, the sulfuric acid is titrated with 0.01 *N* sodium hydroxide in the presence of methyl red indicator.

The efficiency of the Grote absorber was checked by combustion of C.P. sulfur (Table I).

Compounds Containing Carbon, Hydrogen, Oxygen, Sulfur, Nitrogen, Alkali Metals, and Halogens. Sulfur in the presence of halogens and nitrogen cannot be determined by acid-base titration, but it can be determined volumetrically, using standard barium chloride.

The oxidation products which have been absorbed in the 0.1 *N* sodium hydroxide are washed from the Grote absorber in the following manner: The vapor trap is removed and attached to the small rubber pressure bulb, the joint and inside of the vapor trap are rinsed once with distilled water, and the washings forced into a 125-ml. stoppered Erlenmeyer flask. The vertical joint and the upper half of the absorber are rinsed twice with 5-ml. portions of water and the washings and absorption products forced through the frit into the flask by opening the stopcock. Then the lower half of the absorber is rinsed by introducing distilled water through the horizontal joint, shaking, and inverting the absorber, so that the wash water reaches all parts of the lower half of the absorber. The washings are drained into the flask as before. This washing is repeated two times, using about 5 ml. of water each time. If the per cent of sulfur is known to be low, less than approximately 7%, a better titration is obtained if 5 ml. of standardized 0.01 *N* sulfuric acid are added at this point and later subtracted from the total titration.

Five milliliters of saturated bromine water are added to oxidize any sulfite to sulfate. Then 5 ml. of 0.1 *N* hydrochloric acid are added and the solution is boiled on the acid side to expel any excess bromine and to concentrate the volume to approximately 20 ml. It is then cooled in ice water, and 2 drops of phenolphthalein solution are added and enough 0.1 *N* sodium hydroxide to make the solution alkaline. The acidity is adjusted with 0.05 *N* hydrochloric acid until the pink color just disappears. One dipper (approximately 0.15 gram) of the dipotassium rhodizonate indicator is then added, followed by 25 ml. of 95% alcohol and enough water

to make the volume to 50 ml. (±5 ml.). The amber-colored solution is then slowly titrated with 0.01 *N* barium chloride. At the first appearance of a reddish color, the titration should be stopped and the flask vigorously shaken. With continued titration, the reddish brown color deepens and, on addition of 1 to 2 drops of 0.01 *N* barium chloride, changes to a deep cherry red. A premature end point will disappear on shaking in 15 to 30 seconds, while the real end point is stable and will persist even after 10 minutes of shaking.

The detection of this end point has been open to much discussion. The use of light filters first suggested by Hallett (*6*) has been adopted in this laboratory in a modified form.

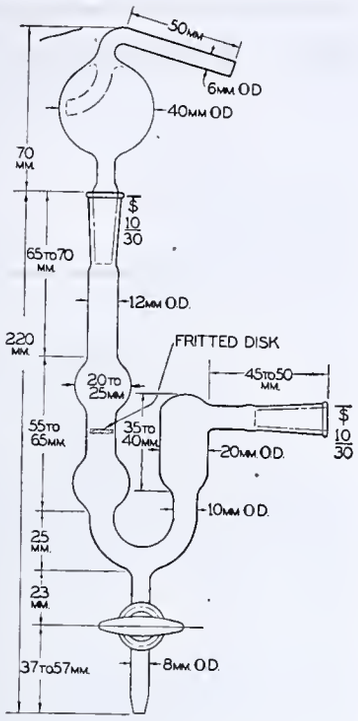

Figure 3. Grote Absorber

This light filter, which is a Wratten filter No. 21 (Eastman Kodak Co., Rochester, N. Y.), is mounted on an opal glass window, 10 × 12.5 cm. (4 × 5 inches) of a small box, 18.75 × 15 × 12.5 cm. (7.5 × 6 × 5 inches), containing a 40-watt blue frosted bulb for illumination. The opal glass area is divided into three parts. The upper third is left uncovered and the lower two thirds are covered, respectively, with one and two thicknesses of the filter material. Thus the flask in which the titration is being carried out can be illuminated by the clear portion and its color, viewed at eye level by transmitted light, compared with the color of one and two layers of the filter. The amber color of the solution being titrated corresponds roughly to a single layer of the filter and persists to within 1 to 2 ml. of the theoretical end point. During the remaining 1 to 2 ml. the color of the solution gradually becomes redder and approximates that of two layers of the filter. The only function of the filters is to indicate the progress of the titration, for as long as the color of the solution corresponds roughly to two layers of No. 21 Wratten filter, the titration should be continued. The end point is a deep cherry red which can be detected without relying on any predetermined exact color match.

In low concentrations of sulfate ion the solubility relation seems to favor the formation of the barium salt of rhodizonic acid, as is apparent from the reddish color of the solution. In concentrations of sulfate less than 5 ml. of 0.01 *N* sulfuric acid, more time and shaking are required to establish equilibrium conditions than in the case of higher concentrations. If the barium chloride solution is run in fairly rapidly with gentle shaking, a red color indicates that an end point is obtained below the stoichiometrical amount required. If allowed to stand for a few minutes, the red color disappears and the original amber color of the solution reappears. The disappearance of the premature end point and the establishment of the equilibrium can be hastened by vigorous shaking. Since the red color produced is due to the formation of the highly colored barium salt of rhodizonic acid, its premature appearance in the presence of excess sulfate ions indicates that the rhodizonic ion is extremely sensitive to barium ions, or that barium rhodizonate forms more rapidly than barium sulfate.

When the end point has been reached, the amount of 0.01 *N* barium chloride used is noted. From this a titration correction value is subtracted. In this laboratory it has been found to be 0.35 ml. by the method described. This correction value is apparently due to the masking of the red color of the barium rhodizonate by the yellow color of the dipotassium salt. It is determined by titrating a known quantity of 0.01 *N* sulfuric acid with 0.01 *N* barium chloride and noting the value to be subtracted to give the correct titration. A considerable amount of gravimetric work has shown that sufuric acid standardized as 0.01 *N* as an acid will serve within the experimental error of this method as 0.01 *N* in regard to the sulfate ion.

In the case of alkali sulfonates or in the combustion of compounds which may leave an ash that would hold back sulfate, the platinum boat containing the weighed sample is placed in a platinum sleeve to prevent any loss of ash by spattering. If desired, the nonvolatile sulfur (as alkali sulfate) may be titrated separately, after dissolving in water, or the alkali sulfate solution may be combined with the volatile sulfur and the two titrated together. The method is applicable to organic sulfur compounds, organic alkali sulfonates, salts of heavy metals of sulfonic acids (except those forming insoluble sulfates in which case some of the sulfur is retained in the ash), and mixtures of organic sulfur compounds with alkali sulfates.

The per cent sulfur is calculated from the following formula:

$$\% \text{ S} = \frac{\text{ml. of 0.01 } N \text{ } BaCl_2 \times 0.1603}{\text{sample (mg.)}} \times 100$$

Since it has been found desirable for best results to have as constant a titration volume as possible, the sample size is usually chosen to give a titration of about 10 ml. Table II relates the per cent sulfur with the sample size to give this titration with 0.01 *N* barium chloride.

The precision and accuracy of the method can be shown from the results of a series of analyses on six purified sulfur compounds, the purity of which was established by complete elementary analysis and by a sulfur determination by the gravimetric Parr bomb macroprocedure. A variety of sulfur compounds was included in this work in order to determine whether the method was independent of the chemical structure. No difficulty was observed in the case of the compounds investigated. The results are listed in Table III.

In the special case of ionizable water-soluble organic sulfates, preliminary work has indicated that the sulfate ion may be titrated directly with barium chloride under the conditions described above.

DETERMINATION OF CHLORINE AND BROMINE

The titration of chloride and bromide with silver nitrate in the presence of adsorption indicators has been described by Fajans and Wolff (*3*), Kolthoff *et al.* (*8*), and Bullock and Kirk (*1*).

Table II. Relation of Sample Size and Sulfur Content

S, %	Weight of Sample, Mg.
0–7[a]	15.0
10	12.0
15	9.0
20	7.0
25	6.0
30	5.0

[a] For the range of 0 to 7% sulfur, 5 ml. of 0.01 *N* sulfuric acid are added and later subtracted from the total milliliters of barium chloride used.

Table III. Precision and Accuracy of the Volumetric Micromethod for Determination of Sulfur

Empirical Formula	No. of Analyses	Per Cent Sulfur: Theory	Parr bomb	Micro volumetric	Standard Deviation
$C_6H_8N_2S$	18	22.86	22.90	22.97	±0.14
$C_{10}H_{17}O_7N_3S$	7	9.90	9.86	10.08	±0.14
$C_9H_{12}O_4N_2S$	7	13.12	13.21	13.16	±0.10
$C_{19}H_{34}O_5N_2S$	7	7.97	7.87	8.07	±0.06
$C_{11}H_{16}O_2N_2S$	7	13.33	13.54	13.41	±0.10
$C_{20}H_{13}O_4N_2SK$	9	7.69	7.61	7.64	±0.07
$C_{12}H_{10}O_2S$	2	14.68	14.86	14.77	...

Table IV. Precision and Accuracy of Volumetric Micromethod for Determination of Chlorine

Compound	No. of Analyses	% Chlorine Theory	% Chlorine Micro volumetric	Standard Deviation
2,4-Dinitrochlorobenzene	6	17.53	17.64	±0.09
1,8-Dichloroanthraquinone	6	25.63	25.61	±0.07
$C_{11}H_{15}NOCl$	6	16.59	16.69	±0.10

Table V. Comparison of the Parr Bomb Macromethod and the Volumetric Micromethod for Determination of Chlorine

Compound	No. of Analyses	% Chlorine Parr Bomb	% Chlorine Micro volumetric	Absolute % Difference
Trianthrimide A	2	1.30	1.08	0.28
Trianthrimide B	2	0.99	1.07	0.08
Trianthrimide C	2	1.05	0.99	0.06
Trianthrimide D	2	1.17	1.18	0.01
Indonaphthol blue A	2	2.26	2.38	0.12
Indonaphthol blue B	2	3.62	3.66	0.04
Indonaphthol blue C	2	8.71	8.89	0.18
Indonaphthol blue D	2	9.88	10.04	0.16

The absorbing solution (3% hydrogen peroxide, reagent grade) is transferred quantitatively from the Grote absorber into a 125-ml. Erlenmeyer flask and concentrated to approximately 10 to 20 ml. Three drops of saturated sodium acetate and 20 drops of the dichlorofluorescein indicator are then added, followed by 10 ml. of acetone. Since dichlorofluorescein tends to become colorless in acid solution, it may be necessary to add more than 3 drops of saturated sodium acetate solution in the analysis of compounds containing several different acid-forming elements. In the case of the bromine determination, it is advisable to add 2 drops of saturated hydrazine sulfate solution to ensure the reduction of any bromate which may have formed during the combustion. The halogen is then titrated with 0.01 *N* silver nitrate. For both the chlorine and the bromine determinations, the indicator used is dichlorofluorescein which gives a rosy pink end point. No correction value is required for this titration.

In the special case of ionizable water-soluble organic chlorides or bromides (except reducing substances), the halide can be titrated directly with silver nitrate after addition of the buffer, dichlorofluorescein indicator, and acetone as described above.

Water-soluble organic iodides may also be titrated directly with silver nitrate, but this procedure is not too satisfactory because of the unfavorable factor inherent in the high atomic weight of iodine.

The per cent halogen is calculated as follows:

$$\% \text{ halogen} = \frac{\text{ml. of } 0.01\ N\ AgNO_3 \times \text{milliequivalent} \times 100}{\text{sample (mg.)}}$$

in which the milliequivalents have the values 0.355 and 0.799 for chlorine and bromine, respectively.

For low concentrations of chlorine and bromine, it is advisable to add a known volume—i.e., 5 ml.—of a 0.01 *N* halide solution before starting the titration. This is necessary in order to obtain a sharp end point, since the mechanism of the indicator requires a definite minimum quantity of the colloidal precipitate on which the color reaction takes place.

The precision and accuracy of this method have been checked by the analysis of pure compounds and also by comparison with results by the gravimetric Parr bomb macroprocedure on impure compounds in which chlorine was present as either a major constituent or an impurity. Tables IV and V show typical results for chlorine.

In a similar manner the precision and accuracy of the method with regard to the determination of bromine were checked by the analysis of pure bromine compounds. The results are listed in Table VI.

Table VI. Precision and Accuracy of Volumetric Micromethod for Determination of Bromine

Compound	No. of Analyses	% Bromine Theory	% Bromine Micro volumetric	Standard Deviation
Benzalacetophenone dibromide	6	43.45	43.57	±0.10
$C_5H_{11}NO_3SBr_2$	6	49.20	49.28	±0.10
$C_{16}H_6O_2N_2Br_4$	6	55.34	55.48	±0.17

DETERMINATION OF IODINE

The iodine is determined by an iodometric method similar to that described by Niederl and Niederl (*9*).

The absorbing solution (5% sodium hydroxide) is transferred quantitatively from the Grote absorber into a 125-ml. glass-stoppered Erlenmeyer flask containing 5 ml. of 20% aqueous sodium acetate solution. A solution of 5 ml. of 10% sodium acetate in glacial acetic acid and 4 to 6 drops of bromine are then added. The excess bromine is destroyed by the addition of 20 drops of formic acid. Solid potassium iodide, 1 to 1.5 grams, and 3 ml. of 1 to 2 sulfuric acid are finally added. After standing for 5 minutes in the stoppered flask, the liberated iodine is titrated with 0.01 *N* thiosulfate solution to a pale yellow color, after which starch solution is added and the titration completed.

During the combustion, some iodine vapor may crystallize out on the cool portion of the Grote absorber. In such cases, an auxiliary heating device should be used, such as a suitably constructed copper shield, or some other method of heat transference.

The precision and accuracy of the method were checked by the analysis of purified iodine compounds (Table VII).

Table VII. Precision and Accuracy of Volumetric Micromethod for Determination of Iodine

Compound	No. of Analyses	% Iodine Theory	% Iodine Micro volumetric	Standard Deviation
4-Iodo-2-nitroanisole	8	45.50	45.42	±0.13
4-Iodo-2-nitroaniline	8	48.09	47.87	±0.11
1-(2-Chloro-4-iodobenzeneazo-2-naphthol)	6	31.07	30.90	±0.15

TRACES OF CHLORINE

Small quantities of chlorine have been determined by a combination of combustion in the Grote apparatus and the estimation of the chloride ion by turbidimetry.

The combustion tube is cleaned with hot dilute nitric acid to remove traces of inorganic chlorides which may have remained in the tube from previous combustions and which tend to volatilize slowly at high temperatures. It is also advisable to treat a new tube in a similar manner. The tube is then tested by a trial run to ensure the absence of any blank. The sample sizes have varied from 20 to 130 mg., depending upon the amount of chlorine present. Three per cent hydrogen peroxide (reagent grade) is used for absorbing the oxidation products. The absorbing solution is transferred to a test tube for turbidimetric comparison with standards prepared from known quantities of 0.01 *N* hydrochloric acid. Three drops of dilute nitric acid, one drop of 20% silver nitrate solution, and water to make the volume up to 20 ml. are added. The turbidity of the sample is compared with standards against a dull black background, preferably under fluorescent lighting. The per cent chlorine is calculated as follows:

$$\% \text{ Cl} = \frac{0.355 \times \text{ml. of } 0.01\ N\ HCl \times 100}{\text{sample (mg.)}}$$

The standard deviation was found to be ±0.01% chlorine when the amount of chlorine was in the range of 0.1 to 0.2% and samples of 20 to 130 mg. were used for the combustion. With a sample weight of approximately 100 mg. the sensitivity was found to be about 0.007% chlorine.

DISCUSSION

Tetrahydroxyquinone (THQ sold by Betz & Company, Philadelphia, Pa.) has been suggested by several authors (*6*) as the indicator to be used in the volumetric sulfur procedure. The literature indicates that this material is the disodium salt of tetrahydroxyquinone diluted with some inert material (*14*). It has been shown by Preisler (*10*) that tetrahydroxyquinone is a misnomer and that the compound is in reality a salt of rhodizonic acid. A sample of the dipotassium salt of rhodizonic acid (kindly furnished by P. W. Preisler of Washington University Medical School, St. Louis, Mo.) was analyzed in this laboratory and found by determinations of carbon, hydrogen, and potassium to be dipotassium rhodizonate. Spectrophotometric curves of solutions of commercial tetrahydroxyquinone (THQ obtained from Betz and Company) and a solution of the dipotassium rhodizonate were found to be similar. In the recent work reported in this paper, the dipotassium rhodizonate has been prepared by the procedure described by Preisler, but in much of their earlier work the authors used the so-called THQ which they now believe was a salt of rhodizonic acid.

In order to determine the most favorable concentration of the system, water–organic diluent, various concentrations of water-alcohol and water-acetone mixtures were tried in titrating a constant volume of 0.01 *N* sulfuric acid. The organic diluents investigated were methyl, ethyl, and isopropyl alcohols and acetone. In one case, the organic diluent was varied from 0 to 45 ml. and in the remaining cases from 5 to 25 ml. The initial volume before titrating was 50 ml. (±1).

The following data were obtained (shown graphically in Figure 4):

The titration cannot be performed in a wholly aqueous medium.

The most favorable composition of the system, water–organic diluent, is 25 ml. of water to 25 ml. of an organic diluent.

The effect produced by increasing the concentration of the organic diluent from 0 to 25 ml. is striking. In each case a sudden break is observed within very narrow limits.

C.P. ethyl, methyl, and isopropyl alcohols were found to be satisfactory diluents. Because of an obscure end point, acetone was not a satisfactory diluent.

At the very beginning of this work it was observed that a titration correction value existed. It became necessary, therefore, to determine this value at various concentrations of sulfate—that is, from 0.00 to 20.00 ml. of 0.01 *N* sulfuric acid. Two cases were considered: (1) the titration of 0.01 *N* sulfuric acid with 0.01 *N* barium chloride, and (2) the same series of titrations in the presence of 5 ml. of 0.10 *N* sodium chloride. The latter case is important because it represents the conditions of an actual analysis.

The following conclusions were drawn from the data obtained:

In both cases, by using the method of titration described, a fairly constant titration correction value existed in the range 5.00 to 20.00 ml. of 0.01 *N* sulfuric acid. The correction value taken was −0.35 ml. of 0.01 *N* barium chloride.

The presence of 5 ml. of 0.10 *N* sodium chloride does not interfere with the titration in the range 5.00 to 20.00 ml. of 0.01 *N* sulfuric acid.

In the absence of 5 ml. of 0.10 *N* sodium chloride, the lowest concentration of sulfate that can be titrated is about 2.00 ml. of 0.01 *N* sulfuric acid, and in the presence of the salt the lowest concentration is about 5.00 ml. of 0.01 *N* sulfuric acid.

In actual practice, the minimum sulfate concentration should not be less than 5.00 ml. of 0.01 *N* sulfuric acid.

The unusual behavior of the system in the range 0.00 to 2.00 ml. of 0.01 *N* sulfuric acid may be due to unfavorable equilibrium conditions. In the presence of 5 ml. of 0.10 *N* sodium chloride this behavior is enhanced and extends through the range 0.00 to 5.00 ml. of 0.01 *N* sulfuric acid.

The data are shown graphically in Figure 5.

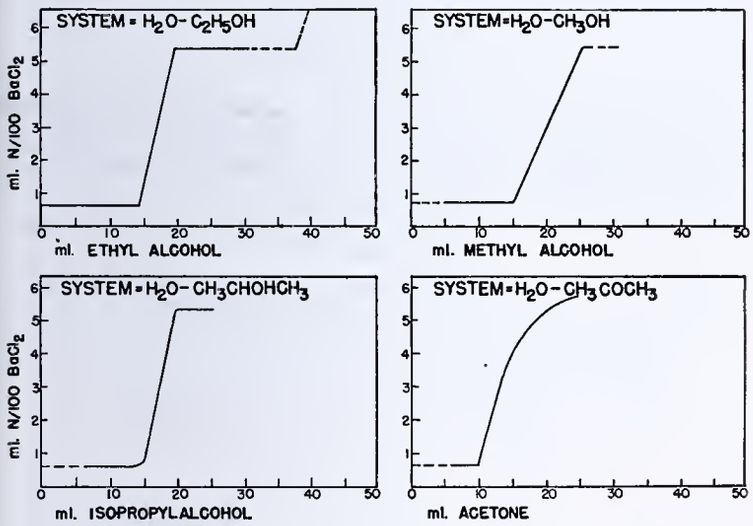

Figure 4. Titrations of Organic Diluents

Titration of 5 ml. of 0.01 *N* sulfuric acid with 0.01 *N* barium chloride. Total volume in each case made up to 50 ml. with water

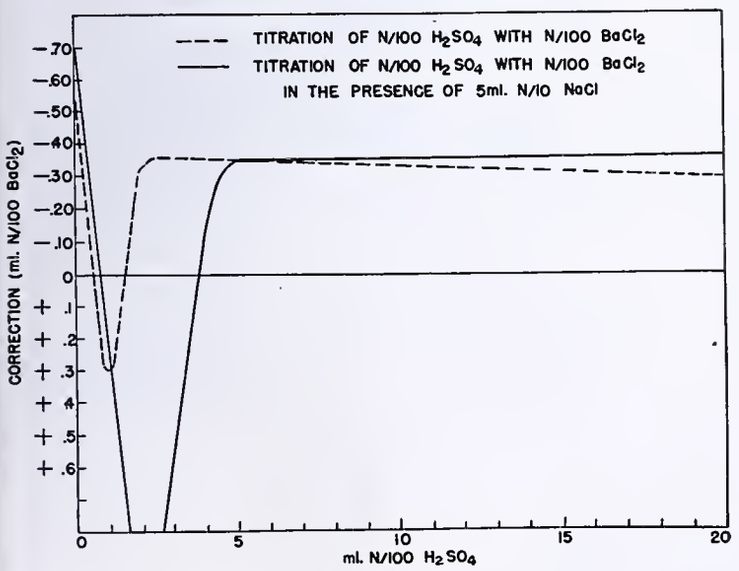

Figure 5. Titration Correction Value

ACKNOWLEDGMENT

The authors wish to acknowledge the suggestions received from the many people who were kind enough to review this paper. Appreciation is also expressed to H. K. Alber of the Arthur H. Thomas Company for the drawings of the apparatus, and to the Corning Glass Works for the several Grote absorbers and combustion tubes furnished during this investigation.

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NOTES ON ANALYTICAL PROCEDURES

New Head for Laboratory Fractionating Columns

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IN DISTILLATION systems, take-off heads which are capable of maintaining a constant reflux ratio regardless of variations in distillation rate and other factors have been described by Bruun (*1*) using a two-way valve, by Carter and Johnson (*2*) using a magnetically operated funnel, and by Snyder and Steuber (*3*) using a U-shaped take-off tube and pressurized receiver which is intermittently vented to the still pressure. None of these devices is satisfactory for the routine laboratory distillation of petroleum products in which appreciable amounts of condensed butane hydrocarbons are to be measured. These low-boiling components tend to vaporize in the take-off line and re-enter the column. The take-off device described here overcomes this difficulty and has the further advantages of high throughput and low holdup.

The feature of this take-off, which is designed for a totally condensing head, is a steel ball bearing encased in glass. This assembly is seated by a joint ground into the throat of a glass funnel leading to the receiver. For alternate use with the head, a double-type condenser utilizing dry ice cooling in addition to the conventional type is described. This condenser gives efficient and complete condensation of C_4 fractions. It avoids the troublesome change from dry ice to liquid coolant, which is encountered in the operation of the usual dry ice head, and eliminates the need for expensive cooling systems.

CONSTRUCTION AND OPERATION

Figure 1 (left) shows the side view of the head, with the take-off mechanism enlarged at center. The steel ball, *A*, encased in glass is shown in position for total reflux, and illustrates the small vapor volume-free space and liquid holdup. The glass funnel, *B*, forms a tight seal for total reflux, the condensate from the drip tip, *C*, flowing over the valve and returning to the column. During take-off a cycle timer connected to an electromagnet, *D* (heavy duty-220-volt alternating current coil 900 ~ resistance), operates to pull the steel ball up to the position of the dotted line and all condensate flows into the receiver. At the end of the take-off period, the valve reseats by gravity and condensate again returns to the column. It has been found desirable, for best operation, to have an indentation blown into the head as shown at *E* to allow closer approach of the magnet core to the valve. Figure 1 (right) shows an alternate type of condenser *F* permitting the use of coolant in center core and in outer jacket. Replaceable attachment *G* on center core facilitates addition of dry ice to the condenser, necessary for condensation of volatile distillates.

Advantages found during operation are: (1) simplicity of operation due to automatic take-off with variable setting for reflux ratio; (2) ruggedness of construction; (3) protection of the steel ball from corrosion and of the distillate from contamination; (4) usefulness for high-temperature distillation because of a small surface area and vapor volume; (5) usefulness for low-temperature distillation because of a positive closure and a variable coolant; (6) high-capacity take-off valve permitting rapid distillation of large volumes; and (7) adaptability to vacuum distillations.

The construction of the apparatus requires glass-blowing skill. However, it can be made readily by a professional glass blower or obtained at a reasonable cost from the Euclid Glass Engineering Laboratory, 6 East 242nd St., Euclid, Ohio. This type of distillation head may be made to fit any size of fractionating column by the proper choice of joint sizes.

ACKNOWLEDGMENT

The authors are indebted to C. J. Schugt of the Euclid Glass Engineering Laboratory and to E. C. Hughes and M. M. Fink of the Standard Oil Company of Ohio Research Laboratory for suggestions in design and for the drawings.

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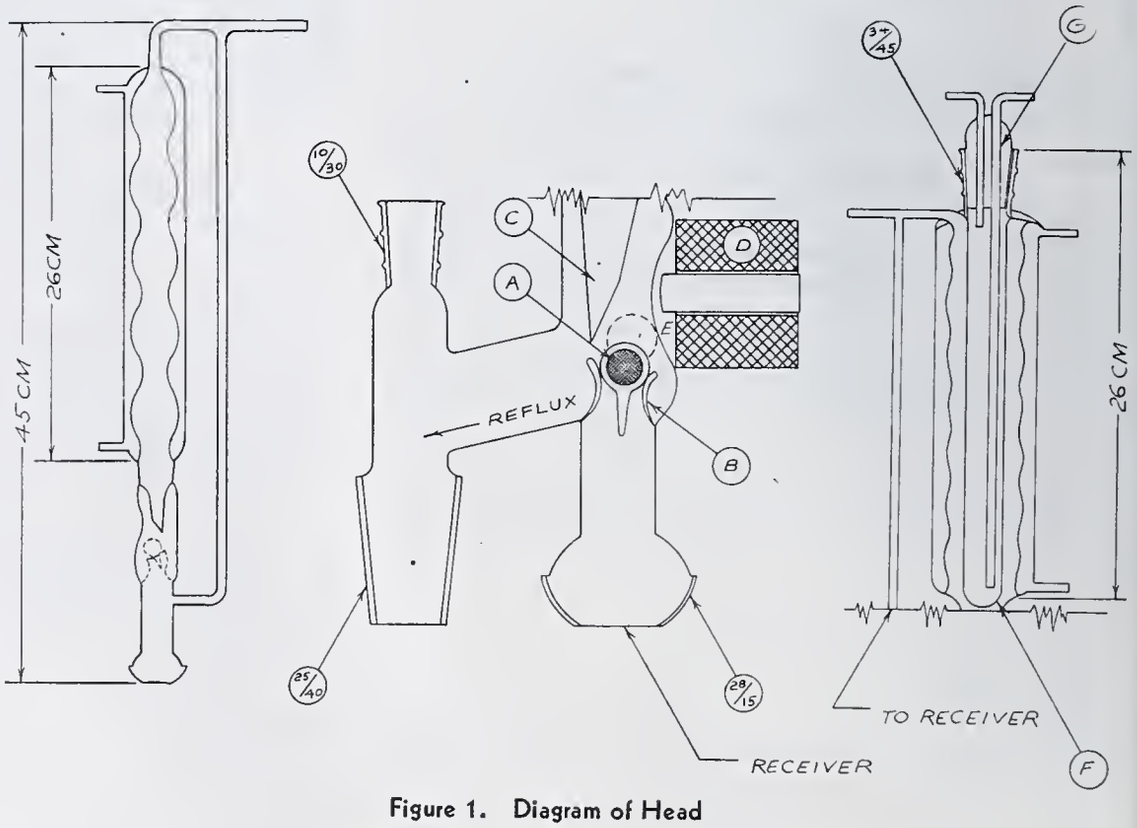

Figure 1. Diagram of Head

Proportional Flow Controller for Liquids

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A PERENNIAL problem in fractional distillations is the accurate control of reflux ratio with a variable throughput. In the laboratory, manual control guided by visual observation of the flow is usually satisfactory. In pilot-plant and larger installations this method is impractical and hence many contrivances have been devised and described for automatic division of the flow in a given ratio. These generally fall into two classes, the intermittent take-off type and the proportional condensing surface type.

Carter and Johnson (*3*) describe a device employing a funnel moved magnetically; Brunn (*2*) uses a two-way valve and capillaries; Podbielniak (*4*) has an intermittently-opening valve seated in the take-off tube; Berg (*1*) describes a proportional condensing area device. Most reflux ratio controllers are difficult and expensive to build, contain delicate moving parts which get out of order, and are intermittent in operation or of such a nature that the ratio may be varied only by stages.

A proportional flow controller free from these defects has been developed in this laboratory and used successfully on a 5-inch diameter column for one year. It operates on the principle of directing a downflow of liquid into a uniform, free-falling sheet or curtain which is split into two parts by a movable divider.

The device consists of an upper and a lower compartment separated by a horizontal plate, as shown in the schematic cross-sectional views of Figure 1. This plate contains a narrow slot through which the entire liquid condensate flows by gravity, the liquid flow being indicated by arrows on the drawings. Just below and parallel to the slot is fixed a vertical sharp-edged guide plate which collects the liquid as a uniform falling film. The guide plate extends down into a movable tray having a vertical divider plate perpendicular to the guide plate; the arrangement of the slot, the guide plate, and the divider plate is shown schematically in Figure 2. The divider plate has a sharp edge, and is notched to fit closely around the guide plate, so that it cuts the falling film into two separate parts, each of which flows out of one end of the tray into the lower compartment. That compartment in turn is divided on the bottom by a vertical plate, underneath the movable tray, which keeps the two liquid streams separated as they spill from the tray.

The dividing tray, which is supported on horizontal rails as shown, can be moved by a rod projecting through a packing gland in the side of the lower compartment, and its position is indicated by a pointer on the rod. The length of the tray, and the position of the divider plate, may be designed so that the flow can be split into any ratio over the required range, which can be anywhere between zero and total reflux.

The liquid head in the upper compartment is determined by the input rate and the slot dimensions, and the only variable in the liquid sheet leaving the slot is its velocity. Thus the instrument is independent of variations in throughput, within the limit set by the maximum available height above the slot. The upper and lower compartments are connected by vent lines, so that unequal gas pressures cannot develop. The vent from the upper compartment also acts as an overflow line into one end of the overflow compartment, in case the rate of flow becomes greater than the capacity of the slot.

The guide plate below the slot maintains the continuity and uniformity of the falling film, and this constitutes the essential feature which makes the device workable. Without it, the velocity of the liquid will usually be insufficient to form a continuous sheet of its own accord. The slot itself is formed of two V-edged plates as shown in Figure 1. The plates were made as shown because the edges must be parallel and straight, and with the soft brass which was used, this shape appeared to offer the best chance of obtaining these qualities. A thin hardened plate,

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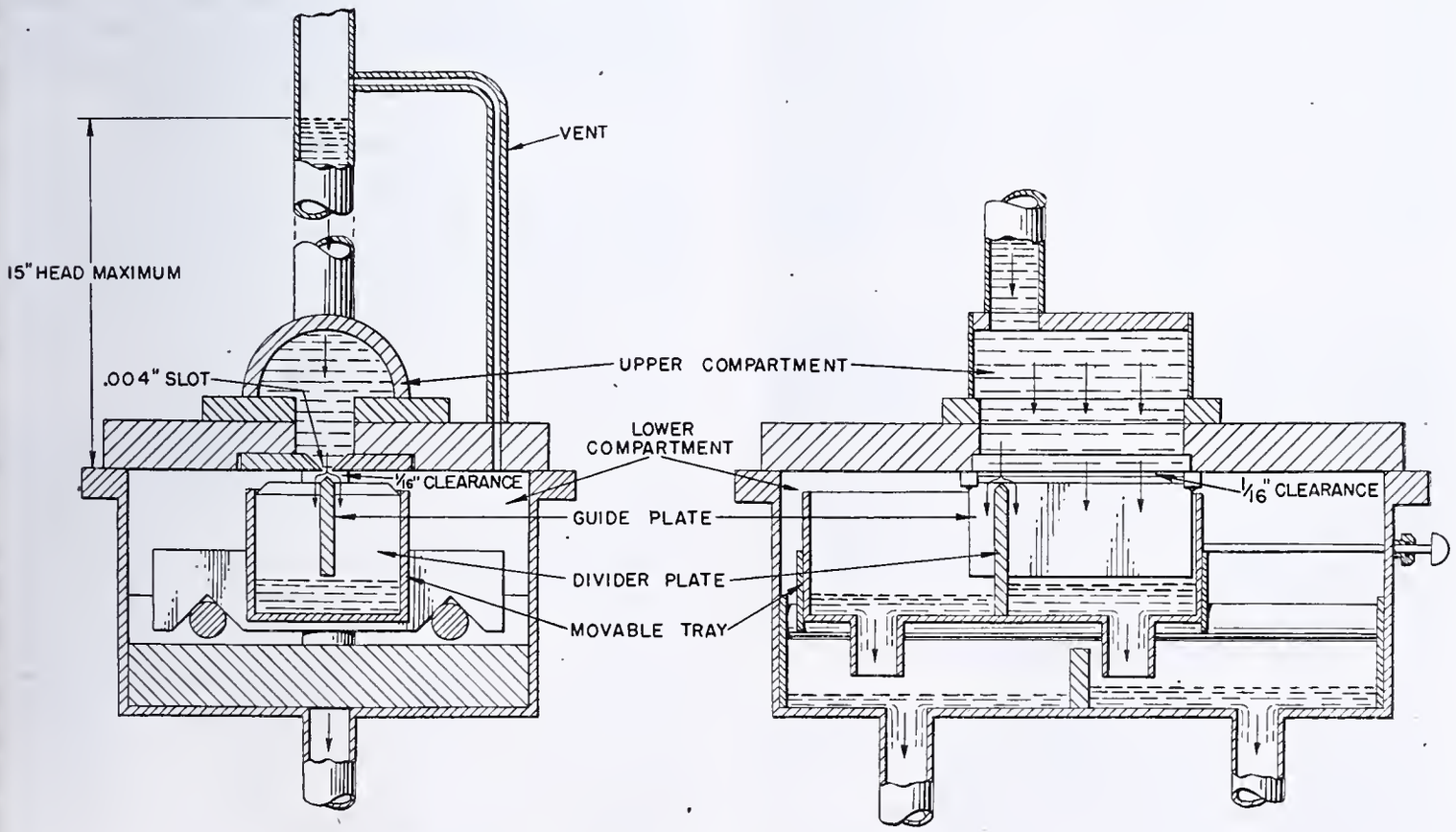

Figure 1. Cross Section of Flow Controller

Left, end view. Right, side view

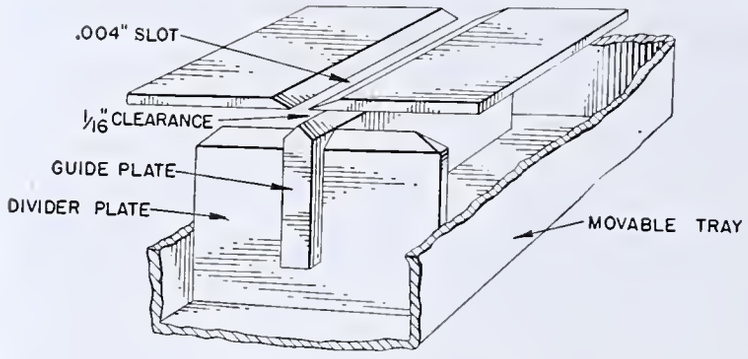

Figure 2. Flow Controller

ground to a sharp edge, would be at least as satisfactory, and probably better than the shape shown in Figure 1. Although the direction of the V as shown does not make the slot self-cleaning, it is necessary to have it as shown in order to feed all the liquid to the guide plate. (A disk-type metal filter with low pressure drop was used on the feed line to remove any scale or sediment from the liquid.) With the exception of the slot, which must be fixed accurately, none of the dimensions are critical, and they may be varied to suit the individual requirements.

The plates are adjustable, so that the liquid head necessary to maintain the proper flow does not become unreasonable. The plates are adjusted at both ends, two feeler gages being used to ensure parallelism. The slot width is determined by the allowable liquid head and the density, viscosity, and surface tension of the liquid. A spacing of 0.0045 inch in a 4-inch long slot is sufficient to carry a flow of 5 to 25 gallons of gasoline per hour at a maximum head of 15 inches, and to provide a continuous sheet of liquid. This slot was tested with gasoline at reflux ratios in the range of 1 to 30. The ratios predicted from the position of the divider were obtained with an accuracy varying from ±1% at a ratio of 1 to ±10% at a ratio of 30, throughout the entire range of 5 to 25 gallons per hour. This degree of precision is entirely adequate for distillation work, but can be further increased by lengthening the slot.

While the present device was designed for reflux control, it is evidently applicable to many installations where it is desired to split a liquid stream into two parts of given, constant ratio. Its advantages include: no moving parts in operation; wide and continuous range of ratios, independent of throughput, pressure, viscosity, etc.; continuous, rather than intermittent, operation with relatively low holdup.

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Improved Apparatus for Karl Fischer Water Determination

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THE need for excluding atmospheric moisture from a Karl Fischer titration is well known; it is equally necessary, for high precision work, to protect the apparatus used from moisture before the titration. Several types of apparatus to accomplish this end have been proposed. Almy, Griffin, and Wilcox (*1*) suggested a stopper drilled to take the buret tip, stirrer, and electrodes, and machined to fit into an ordinary Erlenmeyer flask. Wernimont and Hopkinson (*4*) modified this apparatus by machining the stopper to a standard taper, which with a standard-taper flask gives a more nearly air-tight joint, and replaced the glass stirrer with stainless steel for the sake of durability. More recently, an all-glass apparatus was described by Zerban and Sattler (*5*), in which magnetic stirring is used with a semi-ball-neck flask having a side neck for the electrodes.

The apparatus shown in Figure 1 is an all-glass modification of Wernimont and Hopkinson's apparatus. In use, it is held by a clamp on the upper portion and connected by the standard-taper 10/30 joint to an automatic buret of the overhead reservoir type. The use of a seating-type joint prevents trapping of an air bubble at this point. A radio tube base and a cable connector afford a convenient and positive means of electrical connection to the titrimeter. The one-piece construction of the apparatus eliminates possibility of leakage and adds to convenience of assembly. A glass stirrer is used, since stainless steel is subject to corrosion by the reagent. Less than 5 seconds are required by an experienced operator to remove the flask from the apparatus and unstopper and connect the next flask to be titrated; this feature is also found in the first two types cited. All else being equal, the speed of this operation determines the degree of precision obtainable in the titration.

The dimensions of the apparatus permit use of a 250-ml. Erlenmeyer flask with a 24/40 standard-taper joint, but it could be modified to accommodate other sizes. For small-scale titrations, a vessel in the form of a test tube with a 24/40 joint will permit titration of volumes down to about 5 ml.

A solvent-resistant grease should be used on the buret stopcock and the 10/30 joint, to prevent serious leakage. The 24/40 joint should not be greased, since a slight amount of outward air leakage is necessary to permit addition of the reagent; it is sometimes necessary to grind a slight groove in the male joint to this end. An efficient moisture trap should be used on the air inlet of the buret. (The drying tube usually supplied with the buret is too small to be effective.) The stirrer should be connected to the motor with a short length of soft-rubber tubing to prevent damage due to misalignment. Care should be taken to ensure that the stirrer paddles clear the electrodes. A drop or two of light machine oil in the glass bearing will reduce wear. The electrodes should be kept covered with a spare flask between titrations, that flask being kept stoppered when not in use.

TITRIMETER

Serfass (*3*) has described an electronic titrimeter suitable for detecting end points in conductometric and potentiometric titrations, featuring the use of a "magic eye" electron ray tube for the indicating instrument. McKinney and Hall (*2*) modified the circuit by introducing a voltage doubler, giving a brighter image in the eye. This circuit, although it has the advantage of simplicity and low cost, suffers from two faults which diminish its usefulness. Because it is not isolated from the power line, and because of an inefficient filter circuit, the magic eye image is fuzzy and very susceptible to interferences such as that caused by a stirrer motor. In addition, flickering of the eye is sometimes caused by a ground to the electrodes through the operator's body and a conducting film on the surface of the buret, when the buret is connected to the titration vessel, as in a Karl Fischer titration.

These disadvantages may be eliminated by adding a power transformer, choke, and condenser, in accordance with the diagram shown in Figure 2. A clear, sharp image in the magic eye results from elimination of alternating current component through the improved filter circuit, the by-pass condenser between the grid and cathode of the magic eye tube, and the connection maintaining the heater of the triode at the same direct current potential as the cathode. Although the low potential side of the circuit is grounded, no deflection of the eye is caused by grounding the buret through the operator's body. This effect is eliminated by reduction of the above-ground potential of the

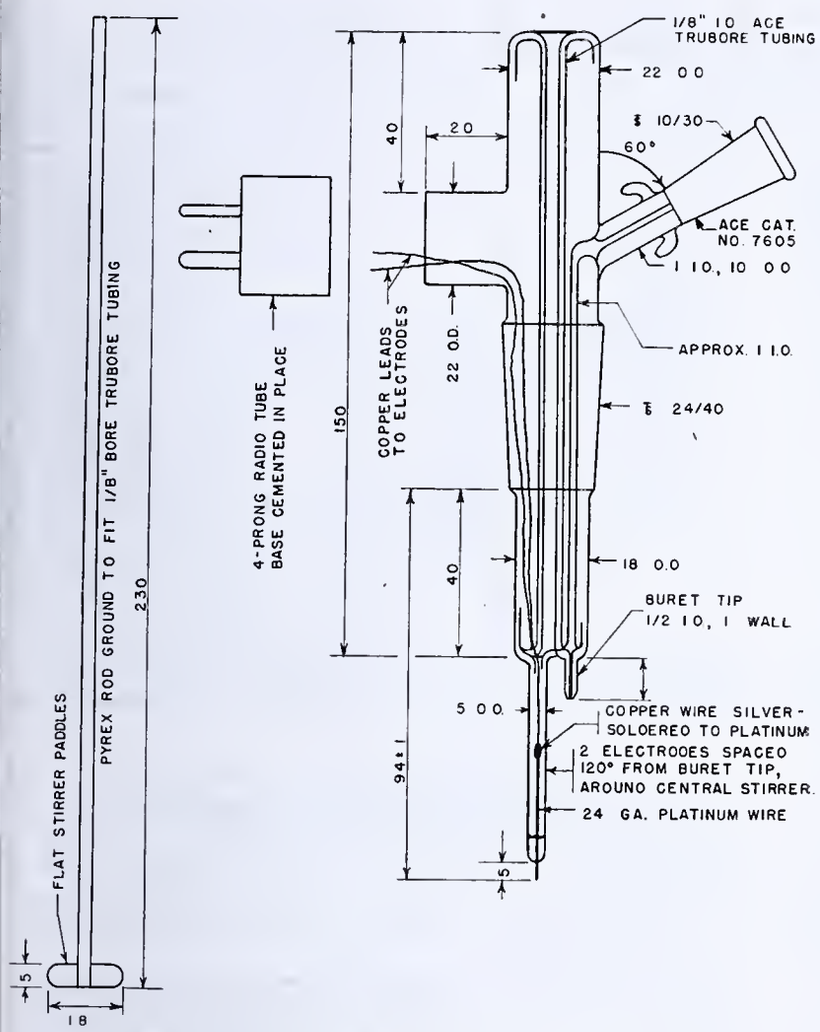

Figure 1. Karl Fischer Electrode-Stirrer-Buret Assembly

amplifier grid to approximately 30 volts, from about 140 in the case of the voltage doubler circuit.

Possibility of greatly increased sensitivity can be introduced by means of the potentiometer in the triode cathode circuit. This is sometimes very desirable in conductometric titrations, where impurities cause a high initial conductivity of the solution being titrated. A similar result could be obtained by replacing the potentiometer with a fixed resistor, and varying the grid bias, as in Serfass' original circuit. However, the desirability of keeping the grid-to-ground potential as low as possible makes the arrangement shown more satisfactory.

Operation of the titrimeter is identical with the original model. Like the voltage doubler model, however, it can be used only on an alternating current power source.

PRECISION

In order to measure the precision obtainable with the apparatus, it was desirable to confine possible deviations to those inherent in the apparatus and the technique involved in its use, as opposed to sampling errors and chemical or physical considerations based on the constitution of the sample. The standardization of Karl Fischer reagent against water was chosen as meeting these requirements.

A solution containing 11.66% by weight of water in methanol was made up in a dropping bottle having a ground-in medicine dropper. This solution, rather than pure water, was used as a standard, to reduce the effect of weighing errors upon precision. Approximately 30 ml. of nearly dry methanol were placed in a 250-ml. standard-taper Erlenmeyer flask, and connected to the titration assembly. The trace of water present was titrated with Karl Fischer reagent until one drop caused the magic eye to remain open for about 30 seconds. (Although this point may be determined with a stop watch, the difference in time required for the eye to close after the next-to-last drop and the last drop is so great as to be readily apparent.) Approximately 1 ml. of the standard water solution was introduced into the flask as rapidly as possible, and titrated as before. The weight of standard solution added was determined by difference.

The results of ten such titrations are given in Table I. The precision is of the same order of magnitude as that obtainable with moderate care in any simple acid-base titration.

Table I. Standardization of Karl Fischer Reagent

Weight of 11.66% Water Solution	Volume of K.F. Reagent	Water	Deviation from Mean
Grams	*Ml.*	*Mg./ml. reagent*	%
0.8613	30.75	3.266	0.0
0.9231	32.90	3.272	0.2
1.0123	36.22	3.259	0.2
0.8252	29.50	3.262	0.1
0.9138	32.61	3.267	0.1
0.9330	33.33	3.264	0.0
0.9561	34.08	3.271	0.2
0.7196	25.72	3.262	0.1
0.8518	30.39	3.268	0.1
0.8181	29.30	3.256	0.3
		Av. 3.265	0.1

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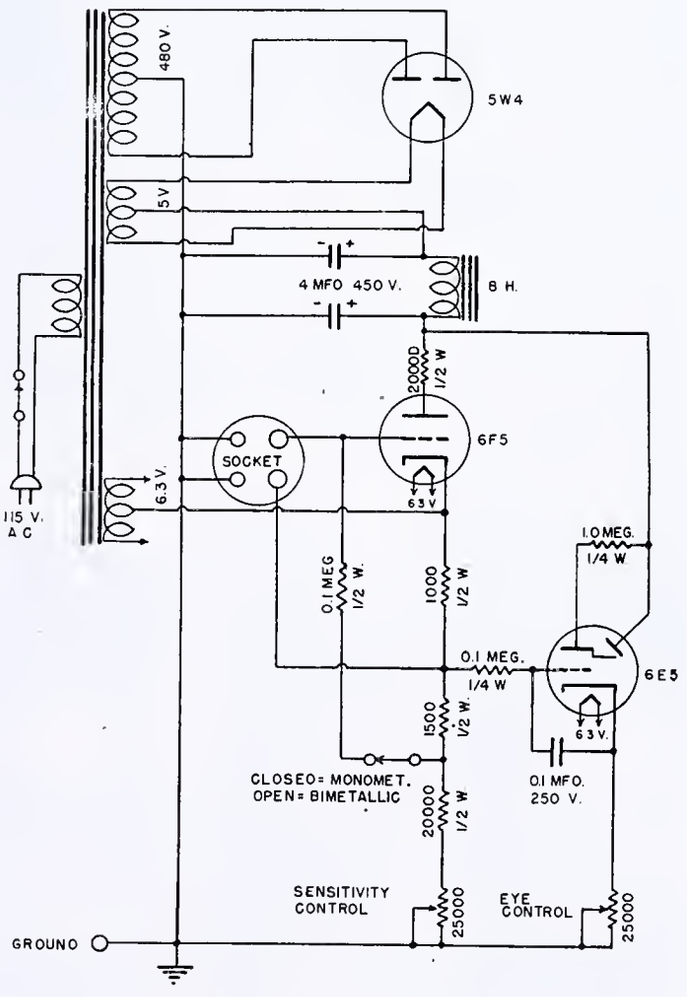

Figure 2. Electrometric Titrimeter

Determination of Chromium by Oxidation with Perchloric Acid

SIGMUND SCHULDINER AND FREDERICK B. CLARDY, Chemical Laboratory, Norfolk Naval Shipyard, Portsmouth, Va.

THE perchloric acid method (*2, 3, 4, 6*) for the determination of chromium essentially consists of oxidation of chromium to the sexivalent state by fuming perchloric acid. The oxidized chromium is titrated with a standard ferrous ammonium sulfate solution to an *o*-phenanthroline end point, an electrometric end point, or a permanganate end point by using an excess of reductant and back-titrating with potassium permanganate solution.

Although the perchloric acid method of oxidation is widely used, the following sources of error have been observed:

1. Loss of chromium by volatilization as chromyl chloride (*1, 5*).
2. Reduction of chromium by hydrogen peroxide formed in the reaction flask (which can be eliminated by rapid cooling of flask and contents).
3. Incomplete oxidation of the chromium due to incomplete heating (least serious of the three).

Experienced analysts have various methods of overcoming these errors and obtaining consistently accurate results. This paper presents modifications of the conventional procedures which have been satisfactorily used in this laboratory for over two years.

EXPERIMENTAL

After experimenting with vertical air and water condensers, the authors found that the sources of error in the perchloric acid method could be overcome by an arrangement of apparatus as shown in Figure 1.

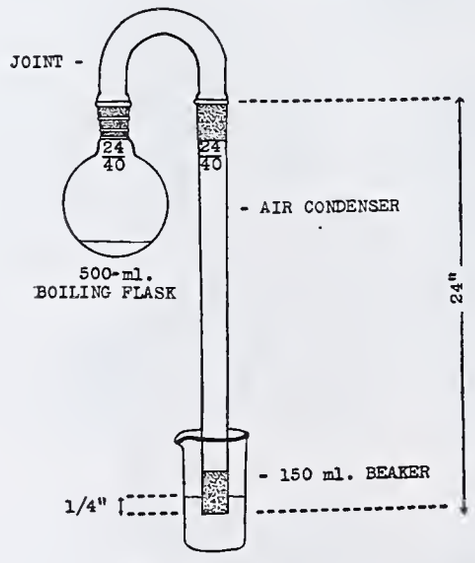

Figure 1. Apparatus

A 500-ml. boiling flask, connected with ground-glass connections by a U-tube to an air condenser, contained the chromium solution and was heated by means of an electric heater. The end of the air condenser was submerged about 0.6 cm. (0.25 inch) in a beaker of water which served to recover any chromyl chloride evolved. The water hydrolyzed the chromyl chloride to chromic acid and hydrochloric acid:

$$CrO_2Cl_2 + 2H_2O = H_2CrO_4 + 2HCl$$

This water solution was titrated and the chromium determined was added to the chromium figure from the reaction flask.

The experimental results in Table I show the amount of chromium that can be volatilized during oxidation with perchloric acid. The first and second determinations of part A, where the chromium was oxidized rapidly by intentionally overheating the reaction flask, show that loss of chromium can be appreciable unless oxidizing conditions are carefully controlled. Tables I and II indicate that, by recovery of chromyl chloride, chromium can be accurately determined. In the modified procedure careful control of the oxidizing conditions is not necessary.

Table I. Recovery of Evolved Chromium

N.B.S. Sample No.	Chromium Present	Chromium Evolved	Chromium Not Evolved	Total Chromium Found
	%	%	%	%
A. Chromium titrated with ferrous ammonium sulfate solution standardized with potassium permanganate solution				
101B	18.50	1.07[a]	17.45	18.52
	18.50	1.81[a]	16.67	18.48
	18.50	0.12	18.36	18.48
	18.50	0.44	18.05	18.49
121a	18.68	0.07	18.63	18.70
	18.68	0.18	18.58	18.76
B. Chromium titrated with ferrous ammonium sulfate solution standardized with N.B.S. sample 101B (sodium chromate dissolved in perchloric acid and reduced with H_2O_2)				
	Mg.	*Mg.*	*Mg.*	*Mg.*
	30.0	0.1	29.7	29.8
	30.0	0.2	30.0	30.2
	30.0	0.0	30.0	30.0
	30.0	0.1	29.6	29.7

[a] Solutions fumed vigorously, intentionally to drive off chromium.

Results shown in Table I, B, were obtained by using hydrogen peroxide to reduce the chromium in a solution of sodium chromate in perchloric acid; the chromium was then reoxidized by the modified procedure.

Table II shows that, within the limits of experimental error, the chromium was completely oxidized. Hence by use of the apparatus and procedure described the chromium factor of the standard ferrous ammonium sulfate solution can be computed stoichiometrically. The chromium results therefore do not depend on an empirical factor but can be derived theoretically.

PROCEDURE FOR HIGH-CHROMIUM STEELS

Place 0.2 gram of sample in a 500-ml. boiling flask with ground-glass connections that can be fitted to an air condenser (Figure 1). Add 10 ml. of hydrochloric acid plus 5 ml. of 1 to 1 nitric acid, and heat until sample is dissolved. Then add 25 ml. of perchloric acid, and heat until the perchloric acid just starts to fume. Connect the reaction flask to the air condenser and place the beaker with about 75 ml. of water in position as in Figure 1. Heat until the reaction flask is cleared of fumes and the perchloric acid refluxes down the sides of the flask. Turn the heater current off and let stand for 2 or 3 minutes. Disconnect the reaction flask and connecting tube from the air condenser and cool the flask rapidly, using first a stream of air and then cold run-

Table II. Results of Chromium Determinations

N.B.S. Sample No.	Weight	Chromium Present	Chromium Found
	Grams	%	%
121a	0.2	18.68	18.66[a]
	0.2	18.68	18.72[a]
	0.2	18.68	18.76[a]
	0.2	18.68	18.76[a]
101a	0.2	18.33	18.37[a]
	0.2	18.33	18.38[a]
101B	0.2	18.50	18.46[a]
	0.2	18.50	18.47[a]
	0.2	18.50	18.55[a]
	0.2	18.50	18.52[a]
111	2.0	0.272	0.274[b]
	2.0	0.272	0.274[b]
72a	2.0	0.655	0.658[b]
	2.0	0.655	0.658[b]
115	1.0	2.16	2.18[b]
	1.0	2.16	2.18[b]

[a] Determined by standardizing ferrous ammonium sulfate solution with standard potassium permanganate solution.
[b] Determined by standardizing ferrous ammonium sulfate solution against N.B.S. samples 101B and 101.

ning tap water. Wash the inside of the connecting tube back into the reaction flask with distilled water and disconnect the tube from the flask. Wash the inside of the air condenser into the beaker of water containing evolved chromium. Pour this solution into the reaction flask. Heat flask and contents until chlorine is completely driven off. Volume should now be about 250 ml. Cool and titrate, following the conventional methods, with standard ferrous ammonium sulfate solution and ferrous *o*-phenanthroline indicator.

The air condenser should be at least 2.5 cm. (1 inch) in diameter and 60 cm. (2 feet) long in order to guard against back-suction of the water into the reaction flask.

After the sample is in solution and the perchloric acid solution is taken to fumes on the hot plate, care should be taken not to oxidize the chromium before the reaction flask is connected to the air condenser as in Figure 1. The solution may be heated to a bright green color but should not turn orange before transfer to the air condenser.

All-glass connections must be used in the oxidizing unit; if fuming perchloric acid comes in contact with organic matter such as rubber, a serious explosion or fire may result.

The reaction flask should be protected from air currents; otherwise the sudden cooling of the flask may cause a suction and some of the water from the water seal may be sprayed into it.

A bank of several oxidizing units can be easily arranged if the work load requires it.

If vanadium is present in the material analyzed, add an excess of ferrous ammonium sulfate solution and then back-titrate with a potassium permanganate solution, omitting the *o*-phenanthroline indicator.

ACKNOWLEDGMENT

The authors wish to express their appreciation to R. S. Gibbs, principal chemist, and Lt. L. D. Wilson, U.S.N.R., laboratory officer, Norfolk Naval Shipyard, for valuable suggestions offered during their review of this paper.

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The views presented in this article are those of the writers and are not to be construed as the official views of the Navy Department.

Apparatus for Quantitative Low-Temperature Vacuum Distillation of Milliliter Volumes

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IT IS frequently desirable to use low-temperature vacuum distillation for quantitative separation of such substances as alcohols, lower fatty acids, and deuterium oxide from nonvolatile materials in small samples. However, this process is inconveniently slow when apparatus incorporates long or complex distillation paths to prevent contamination of the distillate from foaming of viscous solutions, especially those containing protein.

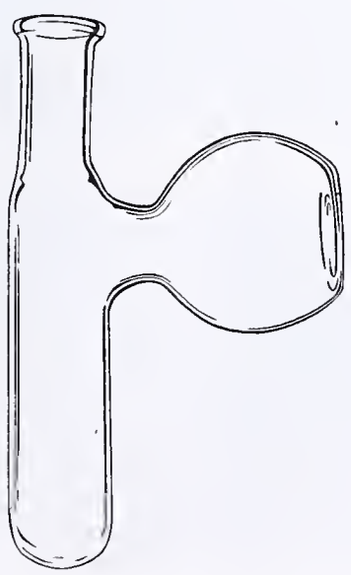
The distillation apparatus shown in the accompanying figure was designed to provide a short distillation path for rapid distillation, while minimizing the chance of contamination from foaming. After trial of several variations in the critical dimensions, this apparatus, which was constructed from parts of a 50-ml. Florence flask and a 25 × 150 mm. test tube, was considered to be of optimum size for the distillation of 1- to 3-ml. samples.

The material to be distilled is introduced into the bulbous side flask by means of a curved-tip pipet. The tube is then closed by a silicone-greased, one-hole rubber stopper carrying a stopcock for connection to a vacuum pump. The side flask is immersed in a dry ice mixture with the tubular portion horizontal. When the contents are frozen, the apparatus is evacuated, sealed by closing the cock, and disconnected from the vacuum line. This whole procedure requires less than a minute. The side flask is then taken from the cold bath and the test-tube portion of the apparatus is immersed in its place, leaving the flask exposed. Distillation is allowed to proceed to completion without artificial heating. Four tubes of the dimensions specified fit conveniently into the mouth of a 0.5-liter Dewar flask.

The apparatus was evacuated by means of a Welch two-stage duo-seal pump which readily reduced the pressure in an empty flask to approximately 1 micron, as judged by the faint blue-gray color produced by a Tesla coil discharge.

With aqueous samples frozen in dry ice, the vacuum appears to be limited by the vapor pressure of the water. During distillation, gases which are trapped in the sample are released and have an appreciable influence on the rate of distillation. Some of this interfering gas can be eliminated by a second evacuation of the apparatus after melting and refreezing the sample. In the case of whole blood the degassing is facilitated by the presence of a small drop of xylene. With a single evacuation, blood, plasma, and urine samples have been distilled at the rate of 0.5 to 1.0 ml. per hour without artificial heating. Comparative distillations made with double evacuation showed an increase in rate of approximately 25% for plasma and saline. Thus, where every saving in time is desirable, the extra manipulation appears warranted.

The quantitative nature of distillation in this apparatus was determined by analysis of distillates from sulfosalicylic acid filtrates of blood to which known amounts of formic acid and methyl alcohol had been added. The distillation of a formaldehyde solution was also studied. Recoveries of formic acid in amounts of 30 to 1000 micrograms per ml. of blood and of methyl alcohol in amounts of 150 to 3000 micrograms per ml. of blood averaged 98 and 101%, respectively, for 9 determinations of each. Solutions containing 1.2 and 3.6 micrograms of formaldehyde per ml. of a mixture of 3% sulfosalicylic acid and 1% sulfuric acid yielded 1.2 and 3.6 micrograms per ml. of distillate. In separate experiments it was found that distillation must be carried to completion in order to obtain the same relative concentrations of volatile constituents in the distillate as in the original sample.



ACKNOWLEDGMENT

The author wishes to acknowledge the assistance of Helen E. Pentz in devising and testing this apparatus.

BOOK REVIEWS

Protective and Decorative Coatings. *Joseph J. Mattiello,* Editor. Vol. V. Analysis and Testing Methods. 662 pages. John Wiley & Sons, Inc., 440 Fourth Ave., New York 16, N. Y., 1946. Price, $7.

In the fifth and latest volume of his series, Dr. Mattiello continues his campaign to place the field of protective and decorative coatings on a firm scientific basis of its own. In answer to the need for systematizing analytical techniques necessitated by the ever-widening variety of new resins and their modifications, he has enlisted the services of several competent author-scientists to contribute to this work.

As with so many other fields of chemical endeavor that have experienced constantly accelerated growth, this industry has amassed data and know-how faster than it could organize and appraise them. Tests that once served as criteria for a given application of a product no longer sufficed to indicate its suitability for a new employment, and so the control chemist gradually lost contact with the firing line of end use. The avalanche of new raw materials and unique applications brought about by the war made it mandatory that the control and research laboratories of the efficient finish company function in close liaison with the actual need. In order to do this, it was necessary to approach the problem from a practical yet academic viewpoint, which is the basic theme of this book.

The first chapter, by R. W. Stafford and E. F. Williams, presents a classification of resins, and means of isolating and studying them by qualitative, physicochemical, and certain special methods. The classification breakdown is: natural resins and their modifications, alkyd resins, aldehyde condensation products, vinyl resins, rubber and rubberlike synthetics, hydrocarbon, and inorganic resins. In addition, the natural and synthetic waxes, carbohydrate, and protein materials are included. A brief discussion of the chemical properties and structure of each member of the above families is given in so far as it bears on their determinations. The second portion of the chapter deals with the analysis of these materials by routine methods and closes with interesting discussion of x-ray radiographic and x-ray diffraction methods of analysis.

Chapter II is concerned with methods for testing and analyzing drying oils. The authors, T. F. Bradley and E. L. Kropa, point out that very few industrial laboratories are concerned with the quantitative determination of a pure fatty acid or its glyceride but rather with the technological values and effectiveness of the material. Viscosity measurements, molecular weight determination, and means for determining the type and degree of unsaturation are discussed, and considerable space is devoted to the use of the thiocyanogen value. The chapter closes with valuable tables of the physical and chemical constants of the commercial fat acids.

A critical appraisal is made in the third chapter of the methods of laboratory testing of metal finishes for outdoor service. The author, S. E. Beck, discusses standardized panel preparation, humidity testing, and the effects of light, temperature, and air circulation on the drying of the films. The sections on statistical evaluation and color are particularly interesting, as are the discussions of abrasion, bending, and flexibility testing.

The spectral characteristics of pigments in the visual and infrared bands are dealt with by V. C. Vesce in three parts: (1) photographic method of measuring visual and infrared reflectances of pigments and pigmented coatings, (2) a spectrophotometric method for the determination of reflectances for the four wave-length bands by the selected ordinate method, and (3) spectral characteristics of pigments. This latter section presents a series of spectrophotometric curves on a large number of synthetic and natural pigments.

The chapter on "Resinography", by T. G. Rochow and R. L. Gilbert, introduces a new approach to resin study that in purpose is related to the analogous fields of metallography and mineralography. Because of the heterogeneous nature of most molded resins it has become useful to study the mode of association of the filler with the embracing resin. In addition, the "metallographic approach" will identify the filler, if it be a metal, and furnish valuable information on the history of fabrication as well. Should the filler be of a mineral nature, either natural or synthetic, a mineralographic approach will yield similar desirable data.

Resinography is also concerned with the structure of the resin or resins involved by studying the resinous phases that may be present and their relationships by difference in refractive index, reflectivity, color, hardness, the effect of polarized light, etc. Indirect methods employing selective staining, selective dissolution, and differential etch patterns are valuable in this respect. The chapter also includes sections on the use of instruments such as the optical microscope and camera and electron microscope in examination of resin specimens.

This book seems to serve well as a clearinghouse and sifting medium for the many diversified but often confusing methods of analysis that have arisen in this field.

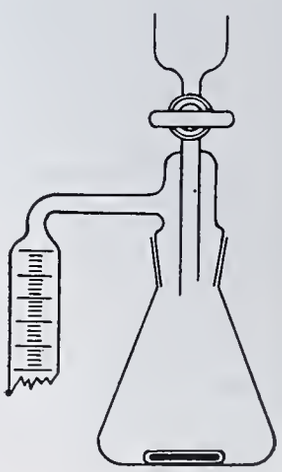
RICHARD L. DEMMERLE

Spectrographic Analysis of Metallurgical Products

The Groupement pour l'Avancement des Méthodes d'Analyse Spectrographique des Produits Métallurgiques has published as a 92-page book the proceedings of its fifth congress, which was held January 22 to 24, 1946. In addition to a report of the meeting and a list of members present, the book includes four technical papers, with discussion: Le Dosage Spectrographique des Faibles Teneurs en Aluminium dans les Aciers Ordinaires et Spéciaux, by R. Castro; Causerie sur les Spectrographes, by H. Tardy; Luminosité Comparée du Spectrographe Zeiss Qu 24 et du Spectrographe Zélande de Jobin et Yvon, by E. Loeuille; and Le Dosage des Traces d'Impuretés dans les Alliages de Zinc au Moyen de l'Etincelle Condensée, by P. Croissant. Copies are available through the Centre de Documentation Sidérurique, 12 Rue de Madrid, Paris 8, France.

Apparatus for Small-Scale Catalytic Hydrogenation—Correspondence

SIR: The continued interest in the apparatus for small-scale catalytic hydrogenation [IND. ENG. CHEM., ANAL. ED., **14**, 907 (1942)], as evidenced by requests for reprints, makes it desirable to describe a modification of the apparatus made shortly after the original article was published. Instead of having the cup and stopcock on the reaction flask, where it caused instability and led to frequent breakage, it was incorporated in the main portion of the apparatus, as indicated in the accompanying diagram. This modification permits the use of any Erlenmeyer flask having an interchangeable ground-glass joint as the reaction vessel.



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Quantitative Analysis of Hydrocarbon Mixtures by Means of Raman Spectra

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THE subject of hydrocarbon analysis by physical methods has become increasingly important in recent years. Of these methods, two make use of the fact that the set of molecular vibrational energy levels is a unique property of each compound. In the method of infrared absorption spectra, a beam of continuous radiation from a hot body is passed through the sample and the transmitted radiation is examined to learn what has been taken out of it. This information provides the raw data for the analysis of the sample. In the method of Raman spectra, essentially monochromatic light is sent into the sample and the scattered light is examined for lines of modified wave length. The analysis of the sample is based on the position and intensity of these modified lines. The literature on this subject is extensive. The following are typical references: (*1*, *2*, *4*, *8*).

Each method has advantages and disadvantages. The present paper describes the authors' technique for applying Raman spectra to the analysis of certain hydrocarbon mixtures and indicates the type of results obtained.

The method described here is not presented as a fully developed method which can be applied with standard equipment directly to routine analysis. However, it is a relatively simple procedure which leads to useful results with liquid hydrocarbon mixtures.

The Raman spectrum of a typical hydrocarbon in the gasoline range consists of a relatively small number of lines whose sharpness depends on the structural type of the hydrocarbon. When two or more pure hydrocarbons are mixed, the Raman spectrum of the mixture is a superposition of the spectra of the individual components without any frequency displacement. The only type of interference which does occur is the accidental overlapping of lines arising from different compounds. With a spectrograph of sufficient dispersion, this is not a serious difficulty for mixtures containing four or five components, and in almost all cases such a mixture can be analyzed by use of Raman lines which are not overlapped. So far as is known, for constant illumination of the sample, the intensity of light scattered by a hydrocarbon in a mixture is directly proportional to the concentration of the hydrocarbon.

Most Raman spectra are accompanied by an appreciable amount of background scattering, caused either by a continuum from the light sources (mercury arcs) or by fluorescence from a minute amount of fluorescent contamination in the sample. The continuum from the mercury arcs can be reduced or practically eliminated by the use of suitable optical filters, or better still, by a choice of mercury arc which emits relatively little continuous radiation. The fluorescence of the sample can often be greatly reduced by a simple bulb-to-bulb distillation without fractionation, or, in more difficult cases, by a distillation over metallic sodium. However, even when efforts have been made to minimize the background, it is usually present to some extent. The only way to overcome this difficulty is to make a suitable correction for the background when the spectrum is subjected to quantitative evaluation.

The method of internal standards has been used, in both emission and Raman spectroscopy, to reduce the effect of variations in the intensity of illumination. A known quantity of some suitable substance is added to the sample and the blackening of the analysis lines is measured relative to that of the lines of the standard. For example, Rank, Scott, and Fenske (*6*) have described the use of 2 cc. of carbon tetrachloride in 25 cc. of sample as an internal standard. The chief objection to this procedure is the undesirable contamination of the sample, which is often needed for other types of work. Even in moderately complicated mixtures there is a considerable probability that the useful lines of the internal standard would be overlapped. The authors have therefore not used internal standards.

The standard procedure of quantitative photographic photometry involves a calibration of the photographic material used by a graded series of exposures of known relative intensity on each plate or film. When the resulting spots are microphotometered, the optical density (defined as the logarithm of the ratio of the light incident on a spot to the light transmitted through the spot) can be graphed as a function of the logarithm of the intensity of the light used for the exposure (for constant exposure time). Then when the density of an analysis line is read from the microphotometer curve for a sample, the corresponding intensity can be read from D–log I curve. This procedure is rather time-consuming.

It has been found possible to evade a great deal of the awkwardness inherent in the photographic method without too much loss of accuracy by a semiempirical procedure involving a calibration for each system based on a few mixtures of known composition made up from compounds of high purity. This procedure also makes it possible to take into account the mutual interference of close-lying Raman lines. Once the calibration has been carried out, the calculations involved are brief and very elementary.

EXPERIMENTAL

The Raman spectra described here were obtained with a Steinheil spectrograph with three glass prisms whose dispersion varies from 6 Å. per mm. at 4000 to 20 Å. per mm. at 5000 Å. (*7*). The spectrograph is enclosed in a thermostated plywood box. The light source used for the earlier part of this work was the GE Type H-2 mercury arc. When this arc operates at its normal temperature, it emits a heavy continuum, which can be reduced by cooling the arc until part of the mercury condenses on the walls. However, under these conditions the light output is annoyingly sensitive to small changes in the temperature of the arc. The General Electric Co. kindly supplied an arc similar to the H-2 but with the mercury content reduced to such an extent that the maximum potential drop is 40 volts. These arcs, desig-

Table I. Calibration for Analysis of Four-Component Sample Using Known Mixtures

Raman Line Frequency, Cm.$^{-1}$	Compound	$I_e°$	Mixture 1 I_e	Mixture 1 P	Mixture 1 $P_{av.}$	Mixture 1 P_c	Mixture 1 R_1	Mixture 2 I_e	Mixture 2 P	Mixture 2 $P_{av.}$	Mixture 2 P_c	Mixture 2 R_2
818	*p*-Ethyltoluene	0.554	0.517	93.3	...	...	...	...	...	...	...	..
806	*p*-Ethyltoluene	0.530	0.527	99.3	92.1	58.1	0.97	...	...	...	...	..
644	*p*-Ethyltoluene	0.421	0.353	83.8	...	...	..	...	...	...	...	..
744	Pseudocumene	1.38	...	...	...	...	..	0.132	9.6	10.0	5.6	0.56
557	Pseudocumene	1.13	...	...	...	...	..	0.118	10.4	...	...	..
520	*m*-Ethyltoluene	0.398	0.215	54.0	54.0	34.1	1.14	0.450	113.0	113.0	63.9	1.07
576	Mesitylene	2.22	0.273	12.3	12.3	7.8	0.78	1.20	54.0	54.0	30.5	1.02
	Totals				158.4	100.0				177.0	100.0	

Raman Line Frequency, Cm.$^{-1}$	Compound	$I_e°$	Mixture 3 I_e	Mixture 3 P	Mixture 3 $P_{av.}$	Mixture 3 P_c	Mixture 3 R_3	Mixture 4 I_e	Mixture 4 P	Mixture 4 $P_{av.}$	Mixture 4 P_c	Mixture 4 R_4
818	*p*-Ethyltoluene	0.554	0.089	16.1	...	...	...	0.265	47.8	...	...	...
806	*p*-Ethyltoluene	0.530	0.079	14.9	15.1	10.1	1.01	0.306	57.7	51.4	32.8	1.09
644	*p*-Ethyltoluene	0.421	0.060	14.3	...	...	..	0.205	48.6	...	...	...
744	Pseudocumene	1.38	0.476	34.5	32.5	21.8	0.73	1.12	80.8	84.8	54.1	0.90
557	Pseudocumene	1.13	0.344	30.5	...	...	..	1.01	88.8	...	...	...
520	*m*-Ethyltoluene	0.398	...	...	...	...	...	0.082	20.6	20.6	13.1	1.31
576	Mesitylene	2.22	2.26	101.6	101.6	68.1	1.13	...	...	...	...	..
	Totals				149.2	100.0				156.8	100.0	

	R_1	R_2	R_3	R_4	$R_{av.}$
p-Ethyltoluene	0.97	..	1.01	1.09	1.02
Pseudocumene	..	0.56	0.73	0.90	0.73
m-Ethyltoluene	1.14	1.07	..	1.31	1.17
Mesitylene	0.78	1.02	1.13	..	0.98

Raman Line Frequency, Cm.$^{-1}$	Compound	$I_e°$	$R_{av.}$	I_c
818	*p*-Ethyltoluene	0.554	1.02	0.565
806	*p*-Ethyltoluene	0.530	1.02	0.540
644	*p*-Ethyltoluene	0.421	1.02	0.429
744	Pseudocumene	1.38	0.73	1.01
557	Pseudocumene	1.13	0.73	0.825
520	*m*-Ethyltoluene	0.398	1.17	0.466
576	Mesitylene	2.22	0.98	2.17

nated as Type H-11, are operated with the mercury completely vaporized and consequently are not appreciably temperature-sensitive. They have proved to be excellent sources for exciting Raman spectra. Six of them are arranged concentrically about the tube containing the sample.

For most of the Raman spectra under consideration here, the Raman tube had a volume of approximately 15 cc.; for the remainder, the sample volume was approximately 6 cc. When the amount of sample available permits, the use of the larger size tube has two advantages: the alignment of the tube with respect to the spectrograph is less critical, and the spectral lines obtained are longer, which makes it easier to select the portion of the lines suitable for microphotometering.

The known aromatic mixtures for calibration were made up from material which was especially synthesized or purified for this purpose. The purities as determined by the freezing point method were all 98% or better and most of them were above 99% (*3*). The usual exposure time for aromatic samples is 75 minutes on Ansco Fluorapid film; for paraffinic samples this is increased to 3 hours because paraffins are poorer scatterers than aromatics. A slit width of 0.1 mm. is used.

The particular Raman lines used for an analysis are those strong lines which have no close neighbors and which show the least overlapping with lines of the other compounds present. Which lines are best often depends on the composition of the sample. It is, of course, necessary to use lines which are neither under- nor overexposed—i.e., lines whose density lies in the linear range of the D–log I curve of the photographic emulsion.

The microphotometer curves required are obtained on a Leeds & Northrup recording microphotometer. The optical density of each Raman line used for analysis is read from the curve as well as the density of the background near each line. The antilogarithm of the background density is subtracted from that of the line density to give an effective intensity of the Raman line (I_e).

The sequence of thought leading to the method adopted for obtaining effective intensities from the microphotometer record was as follows:

1. The exposure of a given line on the negative is assumed to be proportional to the concentration of the corresponding compound in the mixture with constant total illumination.
2. Along the linear portion of the characteristic curve ($D = \gamma \log E$), γ, the gradient, depends only on the emulsion and the development conditions used.
3. Following Pierce and Nachtrieb (*5*), it is desirable to make the background correction in terms of E rather than D. If $\gamma = 1$, then E = antilog D. Since the combination of emulsion and development conditions used gave a value of nearly 1 for γ, this relation was the simplest one to try. The authors have since found that even with somewhat different values of γ they get satisfactory results, presumably because the calibration and the analysis are carried out under the same conditions. A much more rigorous method is the construction of the E *vs.* D curve as Pierce and Nachtrieb have done. In view of other possible sources of inaccuracy, it was not considered worthwhile to adopt their procedure.

Table II. Analysis of Four-Component Mixture

Raman Line Frequency, Cm.$^{-1}$	Compound	I_e	P	$P_{av.}$	P_c	P_{known}	Deviation
818	*p*-Ethyltoluene	0.348	61.6	...	...	...	...
806	*p*-Ethyltoluene	0.352	65.2	61.8	25.5	25	+0.5
644	*p*-Ethyltoluene	0.251	58.5	...	...	...	...
744	Pseudocumene	0.578	57.2	59.0	24.5	25	−0.5
557	Pseudocumene	0.502	60.9	...	...	...	...
520	*m*-Ethyltoluene	0.290	62.1	62.1	26	25	+1
576	Mesitylene	1.244	57.4	57.4	24	25	−1

If the exposure conditions and the photographic processing were strictly reproducible and the assumptions made were valid, the effective intensity as used here could be expected to be a linear function of the concentration, provided the blackening of the Raman lines is kept between the proper limits. Because the actual conditions used are rather far from ideal, even when an effort is made to reach a high degree of constancy, use is made of an empirical averaging process and of the fact that intensity ratios are less affected by variations in exposure and development

Table III. Analysis of C_8 Aromatics

Sample No.	*o*-Xylene Raman analysis	*o*-Xylene Deviation from known composition	*m*-Xylene Raman analysis	*m*-Xylene Deviation from known composition	*p*-Xylene Raman analysis	*p*-Xylene Deviation from known composition	Ethylbenzene Raman analysis	Ethylbenzene Deviation from known composition
R 298B	19	−1	30	0	26	1	25	0
R 393	5	0	10	0	11	1	74	−1
R 395	9	−1	9	−1	76	1	6	1
R 392	77	2	9	−1	5	0	9	−1
R 396	10	0.5	69	−2.5	11	1.5	10	0.5
R 398	4.5	−0.5	4.5	−0.5	5	0	86	1
R 400	22.5	−2.5	55	0	22.5	2.5	..	...
Maximum deviation		−2.5		−2.5		2.5		−1
Av. deviation		1		1		1		1

Table IV. Analysis of C_9 Aromatics

Sample No.	*p*-Ethyltoluene Raman analysis	*p*-Ethyltoluene Deviation from known composition	*m*-Ethyltoluene Raman analysis	*m*-Ethyltoluene Deviation from known composition	*o*-Ethyltoluene Raman analysis	*o*-Ethyltoluene Deviation from known composition	Mesitylene Raman analysis	Mesitylene Deviation from known composition	Pseudocumene Raman analysis	Pseudocumene Deviation from known composition
R 845B	30	0	14.5	−0.5	37	−0.5	18.5	1	..	...
R 812A	25.5	0.5	26	1	..	...	24	−1	24.5	−0.5
R 815A	9	−1	..	...	..	...	63.5	3.5	27.5	−2.5
R 846B	12.5	0.5	..	...	44.5	0.5	43	−1	..	...
Maximum deviation		−1		1		0.5		3.5		−2.5
Av. deviation		0.7		0.8		0.5		1.6		1.5

conditions than absolute intensities. The justification for the procedure is ultimately found in the analytical results obtained with it.

The calibration is carried out, as illustrated in Table I, in the following way:

For each known mixture used for calibration, the effective intensity, I_e, of each Raman line suitable for analytical purposes is divided by the intensity, $I_e°$, of the same line in the spectrum of the corresponding pure compound photographed under the same conditions. The resulting "percentages", P, for each compound obtained from each of its useful Raman lines are averaged, $P_{av.}$ For each mixture the averages $P_{av.}$ are proportionally corrected to give corrected percentages, P_c, which add up to a total of 100%. The ratio, R, between each of the corrected percentages and the corresponding known percentage is obtained and the ratios for each compound are averaged over the mixtures, $R_{av.}$ Then the intensity of each of the analysis lines of each pure compound is multiplied by its averaged ratio, $R_{av.}$, to give a quantity, I_c, which is the intensity of the line corrected for deviations from average conditions.

In setting up a calibration for a series of compounds, it is usually convenient to take the compounds in groups of three, using two or three known mixtures. To extend the calibration to include other compounds which might be found in samples for analysis together with those of a certain calibrated group, it is necessary to use known mixtures containing at least one compound of that group to ensure that corrections are made to the same average condition.

Once the corrected intensities have been obtained, a quantitative analysis is carried out (as shown in Table II for a four-component mixture) by dividing the effective intensity of each analysis line, I_e, by the corresponding corrected intensity, I_c, and averaging the results from the different lines of each compound, $P_{av.}$ These values are proportionally corrected to a total of 100% if all the compounds in the mixture are included in the analysis, P_c.

If the analysis does not include all the compounds in a mixture, it is necessary to determine the total percentage of all those which are included by some independent method. For example, the aromatic portion of an aromatic mixture containing some paraffinic material can be analyzed because the scattering of paraffins is weak compared to that of aromatics, particularly if the paraffinic material contains a number of compounds. In this case, the total aromatic content must be obtained by one of the quantitative methods for determining total aromatics such as acid absorption or silica gel adsorption.

Strictly speaking, the results of an analysis by means of Raman spectra should be expressed in terms of mole per cent. However, the mixtures usually analyzed are made up of closely related or isomeric compounds whose molecular weight and density are nearly constant. When this is true, no significant error is made in expressing the analytical data in terms of volume per cent.

RESULTS

As illustrations of the results obtainable by means of Raman spectra, representative analytical data for several typical mixtures are presented in Tables III, IV, and V. Table III contains results on a number of C_8 aromatic mixtures. In Table IV data on several four-component C_9 aromatic mixtures are given, while Table V exhibits data on ternary mixtures of trimethylpentanes. The average deviation for paraffinic samples is significantly higher than that for aromatic samples. This is a consequence of the fact that the Raman lines of paraffins are weaker and more diffuse than those of aromatics and hence are more difficult to treat quantitatively.

Table V. Analysis of Trimethylpentane Mixtures

Sample No.	2,2,4-Trimethylpentane Raman analysis	2,2,4-Trimethylpentane Deviation from known composition	2,3,4-Trimethylpentane Raman analysis	2,3,4-Trimethylpentane Deviation from known composition	2,3,3-Trimethylpentane Raman analysis	2,3,3-Trimethylpentane Deviation from known composition
R 497	19	−1	31	1	50	0
R 498	30	0	57.5	−2.5	12.5	2.5
R 870A	78.5	3.5	10.5	0.5	11	−4
R 1046C	59	−1	18	−2	23	3
R 1046D	10.5	0.5	15.5	0.5	74	−1
R 1056A	10	0	45.5	0.5	44.5	−0.5
Maximum deviation		3.5		−2.5		−4
Av. deviation		1		1		2

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Operating Characteristics of the Sargent Model XX Visible Recording Polarograph

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The general characteristics of the Sargent-Heyrovský visible recording polarograph are described. Information is presented on its performance in the measurement of half-wave potentials and diffusion currents, and on the operation of the compensator circuit. The performance of the instrument is compared to that of other commercially available recording polarographs.

THE accuracy and reliability of a polarographic analysis depend primarily on the proper control of the various factors that govern the diffusion current, the choice of supporting electrolyte, elimination of interfering substances, and, above all, on an appreciation of the limitations of the method, rather than on the elegance of the electrical instrumentation. An elaborate recording polarograph is not essential to successful applications of polarography; indeed, none of the recording instruments commercially available today is capable of yielding polarographic data of greater accuracy than one can obtain with relatively simple manual apparatus (*3*, *7*, *8*), and some of them are distinctly inferior to the manual equipment in this respect. The sole advantage of a recording polarograph over manual instrumentation is its greater convenience.

There are two types of recording polarographs on the market: (*a*) those that employ galvanometric photographic recording, as in the original Heyrovský-Shikata polarograph (*1*, *3*), and (*b*) those that record the polarogram directly by a pen (or equivalent device) on a moving chart. This paper attempts to present objective information on some of the operating characteristics of the newest instrument of the latter type, the Model XX visible recording polarograph manufactured by E. H. Sargent and Co., Chicago, Ill.

GENERAL

A photograph of the instrument is shown in Figure 1. It is completely self-contained, and to put it in operation it is only necessary to connect a line cord to a 95- to 135-volt, 60-cycle source of alternating current, and to connect another cord to ground. The instrument is robust, with few delicate parts, and is not affected by vibrations. The design of the cabinet leaves nothing to be desired, the numerous controls being mounted on a convenient sloping panel in front of, and below, the recording unit. The recording unit, motors, and chart lights are powered from the alternating current line; self-contained dry batteries are used for the polarographic circuit.

An increasing e.m.f. is applied to the polarographic cell by a motor-driven potentiometer (marked "bridge" on the panel). The resulting current is measured by passing it through a resistance and recording the potential drop, iR, across this resistance with the recording unit. This principle of current measurement has been used very satisfactorily for a number of years in manual equipment (*3*, *7*, *8*). The range of the recording potentiometer is fixed (0 to 5 millivolts) and the current sensitivity is varied by changing the magnitude of the standard resistance.

The continuous chart is 28 cm. wide and it moves at the rate of 3.78 cm. per minute (90 inches per hour). Full rotation of the bridge corresponds to 41 cm. on the chart, and requires 10 minutes 45 seconds. The bridge and chart are driven individually by two synchronous motors, and either can be activated independently of the other or both can be operated synchronously. This good feature, which was also present in earlier models of the Heyrovský-type polarograph, permits the recording of changes in current with time at a constant value of the applied e.m.f.; it is useful in determining rates of reactions involving polarographically active substances—e.g., oxygen.

The bridge is provided with a friction clutch to permit manual adjustment. The attached circular scale is graduated in 100 units, but cannot be read precisely because the index pointer is so far removed from the scale that the parallactic uncertainty is relatively large. The total voltage across the bridge is adjustable from 0 to 3 volts by a variable resistor, and it is indicated on a voltmeter (span potential meter). A potentiometer circuit is also provided for introducing an e.m.f. to the cell independently of the bridge (initial potential control). This enables the starting of a polarogram at any applied e.m.f. from 0 to 3 volts and with any desired voltage (up to 3 volts) across the bridge, and it extends the total range of applied e.m.f. to 6 volts. This is particularly useful when it is desired to make an extended recording of the wave form of a substance with a fairly negative half-wave potential, such as zinc. A switch is provided for changing the zero point of the bridge from its normal end position to the exact center, so that a continuous change from "anodic" to "cathodic polarization" may be obtained. A switch is also present for changing the polarity of the leads to the polarographic cell.

COURTESY E. H. SARGENT AND CO.

Figure 1. Sargent Model XX Visible Recording Polarograph

A "compensator" incorporated in the instrument may be used to send a countercurrent of variable magnitude through the current-measuring resistance to balance out interfering diffusion currents (*2*, *3*, *6*, *9*). The operation of the compensator is discussed in a following section.

The recording unit is a Brown Electronik potentiometer recorder (manufactured by Brown Instrument Co., Philadelphia, Pa.) with a range of 0 to 5 millivolts. The fact that the potentiometer in this recorder is continuously, rather than periodically, balanced causes the instrument to function like a critically

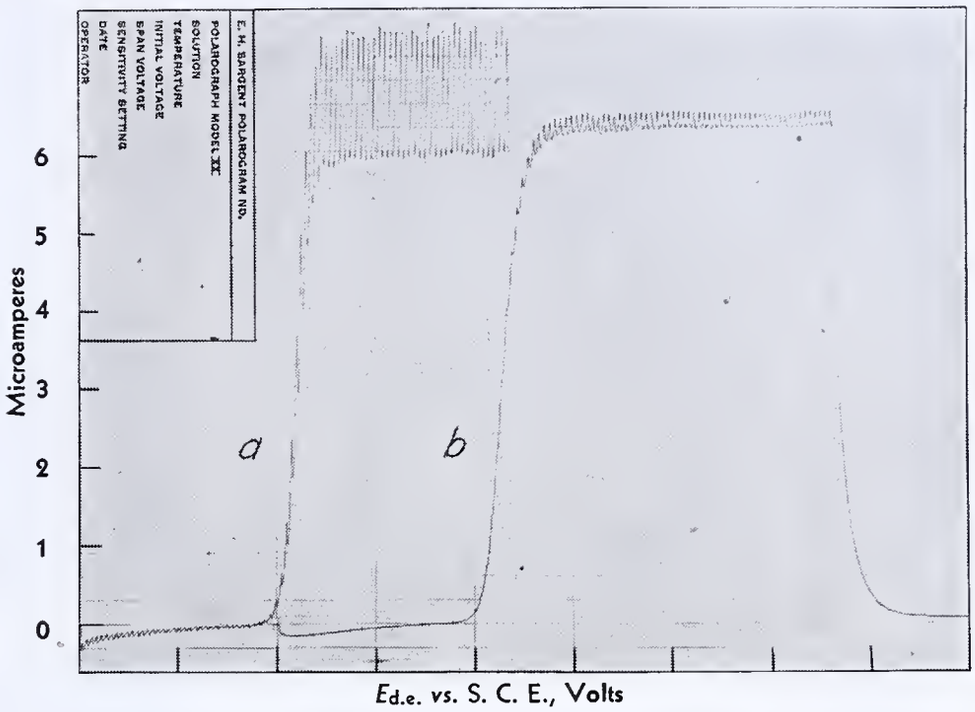

Figure 2. Typical Polarograms of 1 Millimolar Lead Ion in 1 M Potassium Chloride with Different Degrees of Damping

a, damping control in position 1. *b*, damping control in position 5.

damped galvanometer without overshooting or hunting, and thus it is well suited to recording the oscillatory current obtained with the dropping mercury electrode. The recorder has a rapid response, and, provided no external electrical damping is employed, full-scale traverse of the pointer and pen requires only about 5 seconds. Since the e.m.f. indicated by the recorder is the ohmic potential drop across the current-measuring resistance, it is included in the observed values of half-wave potentials, but because the range of the recording potentiometer is only 0 to 5 millivolts the correction will not exceed one half the latter value and is small enough to be neglected in most cases.

The instrument contains an auxiliary circuit, controlled by the zero set rheostat, by means of which the zero position of the recording pen may be adjusted to any position on the scale; like the compensator, this circuit sends a countercurrent through the current-measuring resistors. This adjustment functions satisfactorily, and it is particularly useful when recording polarograms that comprise both anodic and cathodic waves.

Because the accuracy with which half-wave potentials can be measured depends on the accuracy of the span potential and initial potential meters, the latter were checked as follows: The bridge was set to 100 with the outlet leads connected to a potentiometer, and various voltages were set on the meters (by adjustment of the span potential control and initial potential control rheostats) and measured with a potentiometer. The span potential voltmeter was found to be correct within the accuracy with which it could be read (*ca.* ±0.01 volt), but the initial potential voltmeter showed a positive error of 3% over the range from 0.5 to 2 volts. The fact that the applied e.m.f. cannot be measured more precisely limits the usefulness of the instrument for certain research purposes.

The strip chart furnished with the instrument is divided into separate sections, with blank spaces between, the length of each section on the time or voltage axis corresponding to full rotation of the bridge. The voltage axis is provided with ten lines per section, each line corresponding to one tenth of the bridge. The current axis is graduated with millimeter lines. The division into sections is wasteful and inconvenient because care must be taken to start a polarogram well in advance of the end of a section to avoid running into the blank spaces. A chart with continuous markings along the voltage axis would be much more useful. Each section carries in one corner a 7.5 × 14 cm. blocked-in form captioned "E. H. Sargent Polarogram No.", and containing various spaces for noting pertinent data (see Figures 1, 2, and 4). This form has little practical utility and it wastes space. It would also be advantageous for photographic reproduction if the chart were printed in blue lines rather than in green as furnished, and if black ink were used in the pen rather than red.

It is advantageous in recording a series of polarograms to "nest" them together on the chart, especially in preparing illustrations for reproduction. It is awkward to do this with the present instrument, because when the chart is turned back by hand the paper is not taken up on the original roll but accumulates in the back of the chart carriage. If the original roll were provided with a knob, so that it could be turned to take up the slack, this operation would be greatly facilitated. It would also be convenient if some device were provided to support the pen out of contact with the chart to avoid recording during preliminary adjustments of the controls.

It was observed that the instrument, even though grounded, is somewhat sensitive to disturbances by other electrical apparatus in its neighborhood. For example, even with the applied potential switch in the "off" position, a displacement of the recorder zero was obtained when the stirring motor of an adjacent water thermostat was turned on, and also when the thermostat relay operated. (The thermostat and relay also were grounded.) Because of this effect the thermostat was always turned off during the recording of polarograms. It was noted that the "pick-up" effect disappeared when the cell leads were disconnected completely from the instrument, which leads the writer to believe that the effect is due to defective design of the applied potential switch rather than the recording unit.

CELLS

Two types of cells were furnished with the instrument, and in the writer's opinion neither is satisfactory. One cell is of the Erlenmeyer flask type, much used in the early days of polarography but long since obsolete, which requires the use of mercury as anode and cannot be thermostated conveniently. The other cell, which is in the shape of a cylinder with an elaborate ground-glass top, also uses a mercury pool anode and is even less satisfactory than the first, because the anode connection terminates in a radio grid cap sealed on the bottom of the vessel, which makes it impossible to place the cell in a water thermostat. The undesirability of a mercury pool anode, as well as the importance of temperature control, has been discussed elsewhere (*3*). Satisfactory cells and dropping electrode assemblies are not available commercially, despite the fact that these are at least as important units of polarographic instrumentation as the electrical circuit.

The dropping electrode assembly furnished with the instrument, consisting merely of a capillary connected directly to a mercury leveling bulb by rubber tubing, is inadequate for serious work because no provision is made for measuring or reproducing the head of mercury. A stand-tube assembly, preferably with a stop-clock circuit for automatically measuring the rate of flow of mercury (*5*), is most useful for rapid, routine analyses by means of standardized diffusion current constants (*4*). The time is past when polarographic analyses had to be based on frequent, time-wasting "comparison calibrations".

CURRENT SENSITIVITY FUNCTIONS

The sensitivity is varied by adjusting the resistance across which the recorder is connected to record iR drop. Three ranges are selectable by the selector switch marked "Range", with full-scale (28-cm.) deflections corresponding approximately to 0.5, 5,

and 50 microamperes. In addition, a continuously variable resistance controlled by the "sensitivity vernier" permits selection of any desired sensitivity within each range. This is an excellent feature, and the available sensitivity ranges are well suited to polarographic measurements.

The main scale of the sensitivity vernier is graduated from 0 to 100 units, and a vernier scale is attached to facilitate estimation of tenths of a unit. The vernier scale on the author's instrument is rendered rather useless by the fact that the graduations on the main scale are slightly in error at several points; at divisions 10, 40, and 90 the vernier scale spans 8.9, 9.2, and 9.2 divisions on the main scale instead of exactly 9.

The sensitivity functions were determined by connecting accurately known resistances (10,000 ohms for ranges 2 and 3, and 100,000 ohms for range 1) to the cell leads of the instrument, and applying voltage from the bridge to obtain a recorder deflection large enough for exact measurement (*ca.* 200 mm.). The current at each setting was determined by measuring the potential drop across the standard resistance with an external potentiometer and applying Ohm's law (*3*). Denoting by S the sensitivity in millimeter per microampere, and the setting of the sensitivity vernier by V, the functions found for the three ranges were

Range 1. $S = 65 + 5.28\ V$ mm. per microampere
Range 2. $S = 6.0 + 0.538\ V$
Range 3. $S = 0.06 + 0.0533\ V$

With different instruments the constants may be slightly different, but the equations will probably be of the same form. Note that the slopes of the sensitivity lines change by a factor of 10 between each range; the intercepts also change by a factor of 10 between ranges 1 and 2, but by a factor of 100 between ranges 2 and 3.

The sensitivity is conveniently denoted by a symbol like 2-60, signifying sensitivity range 2 and the sensitivity vernier at 60.

An advantage of an instrument of this type over a recording galvanometer is that the sensitivity functions need to be determined only once, whereas a galvanometer requires more or less frequent checking.

DAMPING CONTROL

A damping control is provided to decrease the oscillations resulting from the growth and fall of the mercury drops at the dropping electrode. It functions by introducing capacitors of various magnitudes in parallel with the current-measuring resistors. The fundamental principles of condenser damping in polarographic measurements have been discussed in detail elsewhere (*3, 6*). Five different degrees of damping, corresponding to five different values of the capacitance, are available.

It is essential that the capacitors used in this type of damping have no appreciable direct current conductance, as otherwise the sensitivity will be altered when they are used. To test this point the sensitivity should be determined with steady direct current with the damping control in its several positions. Using steady direct current, and with the sensitivity control in either range 2 or range 3, the sensitivity functions of the author's instrument showed no change when the position of the damping control was varied, indicating that the capacitors were satisfactory.

With the sensitivity control in range 1 it is not feasible to use more damping than that provided by position 1 of the damping control. Even with the damping control in position 1 the recorder is slow in sensitivity range 1, and when the damping is increased the response becomes too sluggish for practical measurement.

Although the sensitivity with steady direct current is not influenced by the position of the damping control, the sensitivity for oscillatory currents is affected. As demonstrated below, diffusion currents recorded by the instrument with the dropping electrode vary with the degree of damping employed (see Figure 3).

ACCURACY OF DIFFUSION CURRENT MEASUREMENT

The most important function of a polarograph is the accurate recording of diffusion currents; therefore, this quality of the instrument was tested with some care. Lead ion in 1 M potassium chloride was chosen as a test substance because its diffusion current constant, $i_d/(Cm^{2/3}t^{1/6})$, has recently been established more accurately than that of any other substance over a wide range of dropping electrode characteristics and by three different methods of measurement (*8*).

Experiments were performed with 1.000 millimolar lead ion in 1 M potassium chloride containing 0.01% gelatin, the experimental procedure being virtually identical with that previously described (*8*). The cell was placed in a water thermostat at 25.00° C., and nitrogen was used to remove dissolved air from the solution. The dropping mercury electrode had a drop time of 3.49 seconds at −0.7 volt *vs.* the saturated calomel electrode, the rate of flow of mercury was 1.50 mg. per second, and the value of $m^{2/3}t^{1/6}$ was 1.615 mg.$^{2/3}$/sec.$^{1/2}$. The diffusion current was measured at −0.7 volt *vs.* the saturated calomel electrode, at a sensitivity of 2-50 (32.9 mm. per microampere), and with the damping control in its various positions from 1 to 5, inclusive.

The polarograms in Figure 2 demonstrate the effect of the degree of damping on the magnitude of the oscillations and on the diffusion current. Taking the average of the oscillations as a measure of the diffusion current, which is the generally accepted practice (*3, 8*), the diffusion current with the damping control in position 5 is 6.5% smaller than with the damping control in position 1.

The gradual decrease in the observed diffusion current constant with increasing degrees of damping is shown graphically in Figure 3; the lengths of the vertical lines represent the magnitudes of

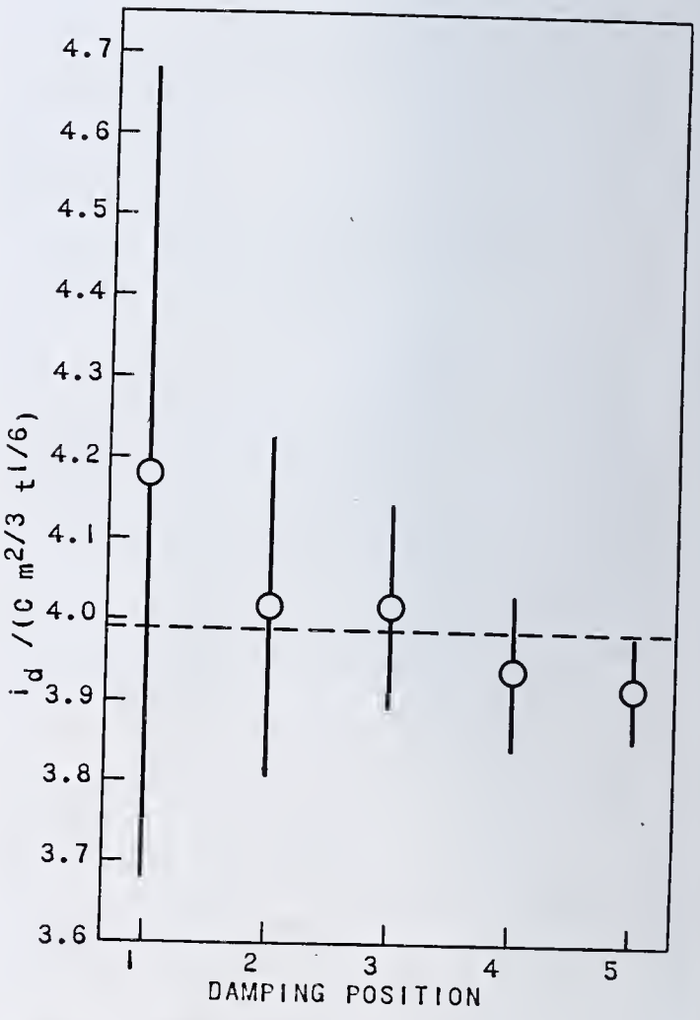

Figure 3. Influence of Degree of Damping on Observed Diffusion Current of Lead Ion in 1 M Potassium Chloride

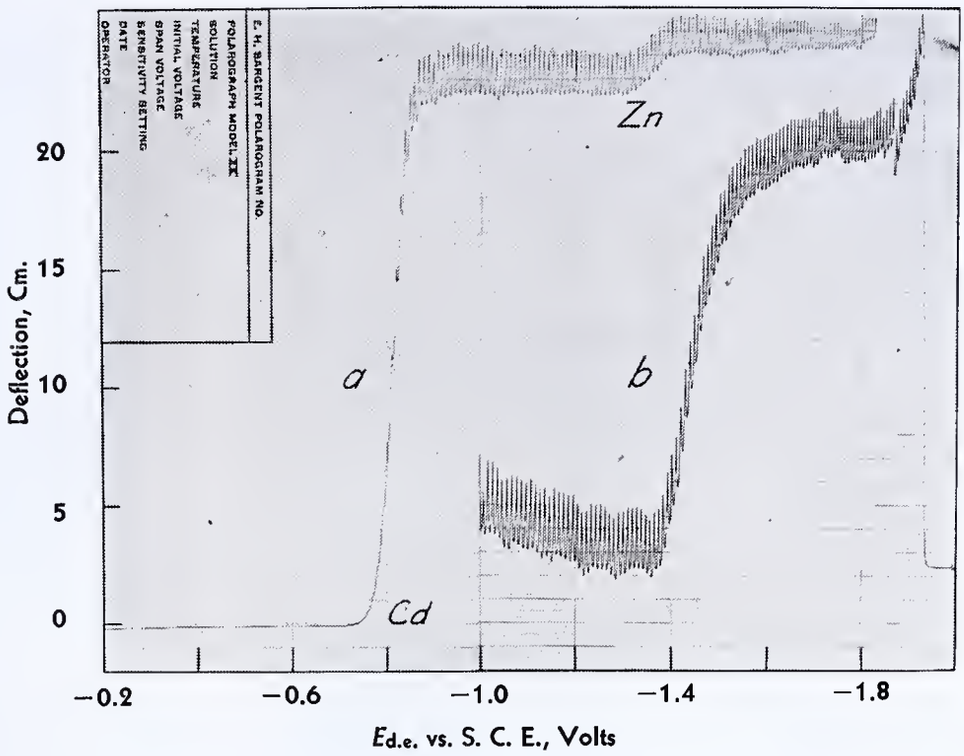

Figure 4. Operation of Compensator in Determination of 0.386 Millimolar Zinc

In presence of 4.65 millimolar cadmium in 1 *M* ammonia–1 *M* ammonium chloride–0.005% gelatin. *a*, recorded at sensitivity of 8.7 and, *b*, at 102 mm. per microampere

the recorder oscillations, and the average of the oscillations is indicated by circles in each case. The horizontal dashed line represents the diffusion current constant found by Lingane and Loveridge (*8*) at the same value of $m^{2/3}t^{1/6}$ used in the present experiments. This value (3.99 ± 0.02) is the average obtained by three different methods of measurement, including manual measurement by the resistance-potentiometer technique, manual measurement with a Leeds & Northrup Type HS galvanometer, and measurement with a Sargent-Heyrovský Model XI photographically recording polarograph. It has been established that these three methods yield results that agree to about 0.5% (*8*). The diffusion current constant measured by the present instrument is considerably too large with the damping control in position 1, but with greater degrees of damping the values agree fairly well (*ca.* ±1%) with the accepted value.

Results exactly similar to those above were obtained with lead in sodium hydroxide solution, and with zinc in ammoniacal medium. It may be concluded that the present instrument records different currents with reasonably satisfactory accuracy, providing that the damping is adjusted so that the magnitude of the recorder oscillations does not exceed about 10% of the average current.

ACCURACY OF HALF-WAVE POTENTIAL MEASUREMENTS

According to the author's experience the half-wave potentials recorded by this instrument tend to be somewhat too large, and the error depends on the degree of damping used in recording the polarogram. For example, the observed half-wave potential of 1 millimolar lead ion in 1 *N* potassium chloride at 25° C. was −0.444 volt *vs.* the saturated calomel electrode when the polarogram was recorded at a sensitivity of 2-50 with the damping control in position 1, and −0.460 volt with the damping control in position 5. The accepted value is −0.435 volt (*3*).

The H-cell used had a resistance of less than 500 ohms, and the half-wave current was 3 microamperes, so the iR drop in the cell did not exceed about 1.5 millivolts. Since the total range of the recorder is only 5 millivolts, the total iR drop across both the cell and current-measuring resistance could not have been greater than about 4 millivolts at the half-wave point on the polarograms. When this correction is applied to the observed values they become −0.440 and −0.456 volt, respectively.

With the damping control in position 1 the observed half-wave potential is in reasonably satisfactory agreement with the accepted value, but in damping position 5 the observed value is about 20 millivolts too large. In sensitivity range 1 the error is considerably larger for a given degree of damping (see Figure 4).

It is inevitable that any type of recording polarograph will show some error in half-wave potentials because of unavoidable lag in the galvanometer or recording unit, and when maximal accuracy is desired, as, for example, in analyzing wave slopes or in obtaining data from which thermodynamic conclusions are to be drawn, the measurements are best made with manual apparatus, or by manual scanning with a recording instrument. The error due to recorder lag with a high degree of damping can be minimized by employing a relatively small bridge voltage in conjunction with a suitable setting of the initial potential, but since the initial potential cannot be set more precisely than about ±0.01 volt at best, this is about the limit of accuracy of the instrument. The accuracy of the instrument is probably sufficient for routine work.

OPERATION OF THE COMPENSATOR

Suppose that a solution to be analyzed contains two reducible substances, A and B, that the half-wave potentials differ by at least 0.2 volt, so that the two waves are well separated, and that the half-wave potential of A is more positive than that of B. Since the wave of A precedes that of B, the sensitivity can be adjusted to the optimum value for determining A, regardless of the relative concentrations of A and B. However, if the concentration of B is much smaller than that of A, and since the sensitivity must necessarily be adjusted to record the total double wave, the wave of B may be so small that it cannot be measured precisely.

In order to determine the minor constituent B without a preliminary separation from A, Hohn (*2*), Thanheiser and Willems (*9*), and Lingane and Kerlinger (*6*) recommended that the interfering diffusion current of A be balanced out ("compensated") by sending an opposing current of equal magnitude through the current-measuring device from an outside source. The sensitivity of the recording unit may then be increased until the wave of B is large enough for convenient measurement. This is the function of the compensator in the present instrument.

The chief limitations of the compensation method are that (1) only the average current is compensated, and not the oscillations, so some means of damping must be employed to prevent the oscillations from becoming enormous when the sensitivity is increased, and (2) imperfections in the diffusion current of A are also magnified and tend to distort the magnified wave of B (*3*, *6*). Hence, the compensation method can only be applied to very well developed waves, and with not too disproportionate concentrations of A and B.

In the present instrument the oscillations are damped by the use of capacitors, as previously recommended with the Heyrovský-type polarograph (*6*). The oscillations can also be kept relatively small by using a very short drop time, but this is very objectionable, because it has been demonstrated conclusively (*8*) that the Ilkovič equation fails seriously when the drop time is smaller than about 1 second.

A typical application of the compensation technique under most favorable circumstances is shown in Figure 4. Curve *a* was obtained with a solution containing 4.65 millimolar cadmium and 0.386 millimolar zinc in a supporting electrolyte composed of 1 *M* each of ammonium chloride and ammonia, 0.005% gelatin, and 0.1 *M* sodium sulfite to remove oxygen. This case is a most favorable one because the half-wave potentials of the ammonia complexes of cadmium and zinc differ by 0.55 volt, the cadmium diffusion current is very well developed, and the concentration of cadmium is only twelve times larger than that of the zinc.

Curve *a* was recorded with a sensitivity of 2-5, and with the damping control in position 5 to provide maximal damping. The drop time was 2.80 seconds at −1.2 volts, an optimum value. Curve *b* was recorded by increasing the sensitivity to 1-7 (a factor of 12 compared to curve *a*), and adjusting the compensator to balance out the diffusion current of the cadmium. Curve *b* was started at −1.0 volt.

The author is not at all convinced that the magnified zinc wave (curve *b*) can be measured with any greater real accuracy than the original zinc wave in curve *a*, which, although small, is very clearly developed. Accurate measurement of a diffusion current is more dependent on the quality of a wave than on its mere magnitude. The measurement of the magnified zinc wave is complicated by the magnification of the normal decrease of the cadmium diffusion current with increasing potential, by the magnification of imperfections in the cadmium diffusion current, which render extrapolation uncertain, and also by the fact that with sensitivity range 1 and the damping control in position 5 the recorder is so sluggish that it lags very considerably and the diffusion current of the zinc is hardly reached before the final current rise. This lag also causes a spurious shift of nearly 0.1 volt in the half-wave potential of the zinc. Curve *b* could not be recorded with less damping because the oscillations were inconveniently large.

According to the writer's experience, claims that the compensation technique largely eliminates the need for chemical separations are much too optimistic.

SUMMARY

The writer would list the following as significantly good qualities of the Model XX polarograph in order of relative importance: (*a*) the instrument is robust and immune to vibrations, (*b*) the current sensitivity functions need be determined only once, eliminating periodic calibration, (*c*) the sensitivity is continuously variable, (*d*) diffusion currents may be recorded as a function of time at a constant applied e.m.f., and (*e*) the polarogram is recorded visibly.

On the debit side may be listed: (*a*) observed diffusion currents vary with the degree of damping employed, (*b*) half-wave potentials vary with the degree of damping and may be significantly in error under certain operating conditions, (*c*) the compensator appears to be of little practical value, and (*d*) the cells and dropping electrode assembly supplied are inadequate.

A detailed discussion of the comparative characteristics of various commercially available polarographs would be beyond the intended scope of this paper, but some general points might be mentioned. There are only two other recording polarographs on the market in this country, the Model XII Heyrovský polarograph manufactured by the E. H. Sargent Co., which employs photographic recording, and the Electrochemograph of the Leeds & Northrup Co. which is a visible recording instrument incorporating a modified Micromax potentiometer recorder. The Model XX is the most expensive of the group, its price of $1500 being nearly three times that of the Model XII and twice that of the Electrochemograph.

The Model XII is by far the simplest to operate and, according to the writer's experience, it is the most reliable and generally useful of the three instruments. It has been demonstrated conclusively that diffusion current constants obtained with this instrument agree exactly with those measured by manual methods (*9*), and on the basis of numerous data it is known that the instrument also measures half-wave potentials accurately. These are the two fundamentally important functions of a recording polarograph, and they are discharged more satisfactorily by the Model XII instrument than by either the Model XX or the Electrochemograph.

The purported inconvenience of developing the photographic record from the Model XII polarograph is more illusionary than real. In actual practice, as in most other methods of analysis, the time spent in the final instrumental measurement is usually only a small fraction of that consumed in the preliminary chemical operations required to prepare the solution, and therefore whether the polarogram is recorded photographically or visually is of minor significance. The writer is not convinced that the total time spent in analyzing a series of a dozen similar samples with the Model XX instrument would be significantly less than with the Model XII, if one includes all operations from the weighing of the sample to the recording of the final result.

A real disadvantage of the Model XII, which is shared to some extent by the Electrochemograph, is sensitivity to vibration, and in certain locations in industrial laboratories special vibration-free mounting may be necessary. Under such conditions the Model XX, which appears to be completely indifferent to even severe vibration, becomes the logical choice.

In both the Model XX and the Electrochemograph the visible recording feature has been achieved by sacrificing a great deal of simplicity, and both instruments have more complex personalities than the Model XII. From the viewpoint of the academician interested in the instruments for their own sake this makes them more interesting, but one who planned to put the instruments into the hands of sketchily trained technicians might well have a different opinion. The Electrochemograph is the more complicated of the two instruments, and the writer's experience with it has been less satisfactory than with the Model XX. The periodically balancing recorder in the Electrochemograph possesses more lag, and seems to be less adaptable to polarographic measurements, than the continuously balanced recorder in the Model XX.

A unique advantage of both the Model XX and the Electrochemograph is that they can be used for recording changes in diffusion current over an extended period of time at a constant applied potential. Hence these instruments may be valuable in kinetic studies for following slow reactions involving polarographically active substances. They should also be useful for certain control purposes, such as recording changes in oxygen concentration of solutions over long periods of time.

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Automatic Determination of Aniline Point of Petroleum Products

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An apparatus is described for the automatic determination of aniline point of petroleum products. The basic principle involved comprises the use of the electric eye as a substitute for visual observation. The test sample is internally heated or cooled, the temperature is maintained uniform by stirring, and temperature equilibrium is automatically accomplished by a relay system. The relay system which controls the heating or cooling is activated by directing a light beam through the sample on a photocell, the light beam being interrupted periodically as the sample clears or clouds with slight temperature change. The flashing of a lamp bulb indicates when equilibrium temperature is reached.

THE A.S.T.M. defines aniline point (*1*) as "the minimum equilibrium solution temperature for equal volumes of aniline and petroleum product".

Many attempts have been made to adapt the test to dark-colored products. Donn (*3*) proposed determining the temperature at which a break occurred in the viscosity-temperature curve. Van Wijk and Boelhouwer (*8*) proposed the detection of a change in transparency to infrared radiation by the use of a thermopile, while Matteson, Zeitfuchs, and Eldredge (*6*) described an apparatus also employing infrared radiation but using a photocell and microammeter. Williams and Dean (*9*) suggested a "circulating test tube" with visual observation through a 2.0-mm. layer. Carr and Agruss (*2*) and Madsen (*5*) recommended an apparatus for determining equilibrium temperature visually through a film of the sample.

Geddes (*4*) in order to overcome the difficulty and hazard involved in determining high aniline points of highly paraffinic lubricating oils proposed substituting *N*-methylaniline for aniline. Data indicate that *N*-methylaniline points are approximately 77° C. lower than aniline points.

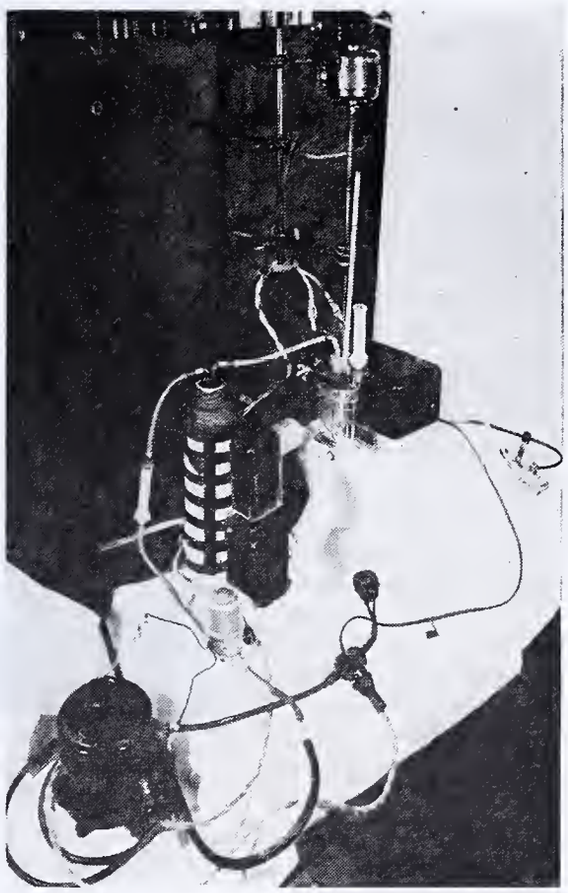

Figure 2. General View of Apparatus

The purpose of this paper is to describe an apparatus that determines aniline points automatically and has the definite advantage that at equilibrium temperature the thermometer mercury column remains practically constant, varying usually not more than 0.2° C.

APPARATUS

1. Air-drying tubes, 12.0 cm. × 17 mm. in outside diameter, filled with 8-mesh Drierite.
2. Bunsen valve 2.5-inch piece of gum tubing, 9 mm. in outside diameter, 5-mm. in inside diameter.
3. Indicating lamp bulb, ordinary, frosted.
4. Knife-switch, double-pole, single-blade.

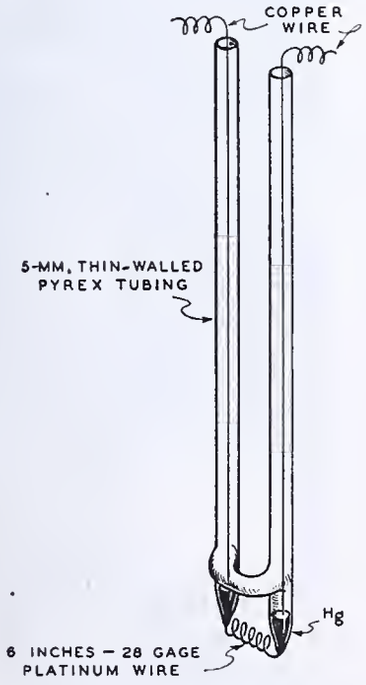

Figure 1. U-Tube for Conducting Cold Carbon Dioxide–Air Mixture

Contains heating unit

5. Light source, General Electric Catalog 98 × 264 Mazda 1133, 32 C, 6- to 8-volt. 3.8-cm. condensing lens. General Electric transformer, Catalog 71G620 KVA 0157, 50/60-cycle 115-volt to 4.8-volt.
6. Photocell and relay Photoelectric relay CR7505 K100-GZ, 115-volt 50/60-cycle. Connections K-6919764, phototube 923 (2nd tube ZG-479).
7. Platinum heating unit, 15.0 cm. of No. 28 gage (see Figure 1).
8. Plexiglas air-jacket, made from 0.15-cm. sheet.
9. Sample container, modified Thiele melting point tube. Over-all length, 17.8 cm. Main body: length 8.25 cm. diameter 2.54 cm. Top opening, 3.5 cm. Charging opening, approximately 12 mm.

Circulating side arm, 12-mm. outside diameter. Pyrex tubing, approximately 7.6 cm. over-all length, with flattened area of approximately 2.0-mm. inside diameter and with a centered bulls-eye of about 0.6-cm. diameter, the sides of which are just close enough together to allow a thin layer of sample to circulate between.

Interchangeable ground-glass joints have been tried, but cork has proved satisfactory.

10. Solenoid valve, General Electric CR9503,115-volt.
11. Stirrer, steel, 0.3-cm. diameter, 41.6-cm. shaft, equipped with 1.3-cm. vertical blades.
12. Stirrer-motor equipped with rheostat.
13. Thermos bottle. Quart size filled with pieces of dry ice stoppered with a rubber equipped with 5- to 6-mm. Pyrex tubes, outlet tube extending to bottom. Wrapped for protection against breakage.
14. Thermometer, A.S.T.M. aniline point. It is believed that one thermometer of suitable range can be used in view of constancy of the temperature at equilibrium point.
15. U-tube. 5.0-mm. Pyrex tubing for circulating cold air and

containing heating unit (Figure 1). Thin-walled tubing is necessary for efficient cooling.

Figure 3. Close-Up of Container

PROCEDURE

For Aniline Points above Room Temperature. Measure sufficient sample aniline through the charge inlet into the sample container to ensure thorough mixing through side arm. Place thermometer in position and attach to stirrer bearing with an elastic band to prevent vibration. (Photocell relay circuit, indicating lamp bulb, and Variac are connected in parallel.) Switch on light source and photocell circuits and set knife switch blade in position, so that the relay will break the heating unit circuit when the solution is clear. Start the stirrer and adjust speed until the sample circulates through the side arm without drawing in air bubbles. Set Variac, which controls the heating unit, to 3 to 5 volts and turn on switch. Focus light beam on flat area of container side arm and direct towards photocell. Heating continues until the sample clears, thus allowing transmittance of light beam to the photocell and thereby breaking the heating circuit. The sample cools immediately, clouds, and shuts off light beam to photocell, and relay is activated to heat-on position. These clearing and clouding cycles are uniform and of very short duration and are indicated by the flashing lamp bulb.

Temperature is maintained practically constant, holding usually within 0.2° C. Adjust Variac, if necessary, according to the difference between test and room temperatures.

Shut off electric circuits upon completion of test. Drain sample by opening stopcock. Rinse sample container thoroughly with suitable solvent (acetone, benzene, or mixture of both) through charge opening and then apply vacuum through stopcock to dry container.

For Aniline Points below Room Temperature. The presence of water seriously interferes with the accuracy of aniline point determinations (7). In determining aniline points occurring below room temperature, air will be drawn into the sample container as cooling takes place. This might be serious in locations where excessive humidity is common. Therefore, the following precautions should be observed.

Make certain that the stopper and fittings are tight. Fit a drying tube into the charge opening by means of a rubber stopper, after sample and aniline are in the container, to act as dry-air breather opening. Insert the thermometer into position through a snug-fitting rubber sleeve.

Replace heating unit circuit with solenoid-activated cooling circuit and set Variac to 115 volts. (Solenoid, photocell relay circuit, and indicating lamp bulb are in parallel.) Insert Bunsen valve in air line between laboratory air supply and solenoid valve. connect the outlet of the solenoid valve to a drying tube, which in turn is connected to the thermos bottle containing the dry ice; make final connection to U-tube in sample container. Turn on the air until an excess blows through the Bunsen valve. Switch on light source, photocell circuit, and stirrer, and reverse knife switch blade in position so that relay breaks circuit to solenoid when the sample clouds. Cooling continues until clouding occurs, thus interrupting the light beam to the photocell which breaks circuit to solenoid, closes valve instantly, and diverts the air through the Bunsen valve. The sample warms immediately and clears, again allowing transmittance of the light beam to the photocell, which activates relay to close solenoid circuit and thereby opens air valve. These clearing and clouding cycles are indicated by the flashing of the lamp bulb and produce a practically constant equilibrium temperature.

Shut off electric circuits and clean container as directed above.

Table I. Aniline Point Determinations

Sample No.	Description	Results: Proposed automatic ° C.	Results: A.S.T.M. ° C.
1	65 ml. of benzene diluted to 100 ml. with *n*-heptane	−11.15, −11.4	−11.4
2	60 ml. of benzene diluted to 100 ml. with *n*-heptane	−3.4, −3.6	−3.7
3	50 ml. of benzene diluted to 100 ml. with *n*-heptane	10.05, 10.2	10.1, 10.1
4	43 ml. of benzene diluted to 100 ml. with *n*-heptane	20.3	20.2
5	Gasoline cut from still	30.8	31.0
6	34 ml. benzene diluted to 100 ml. with *n*-heptane	31.2	31.3
7	Light-colored naphtha	35.7, 35.7	35.9
8	Gasoline cut from still	39.1	39.2
9	Gasoline cut from still	40.8	40.8
10	Light-colored naphtha	49.6, 49.7, 49.6	49.7
11	Diesel fuel	62.0	62.0
12	*n*-Heptane	68.9, 68.9, 69.0	69.1, 69.2
13	Light-colored naphtha	77.6, 78.0, 77.7	77.8
14	Lubricating oil, NPA color 3	111.0	111.2

Table II. Mixed Aniline Point Determinations

Sample No.	Description	Results: Proposed automatic ° C.	Results: A.S.T.M. ° C.
7	Light-colored naphtha	53.2	53.4
10	Light-colored naphtha	59.8	60.0
13	Light-colored naphtha	73.0	73.2

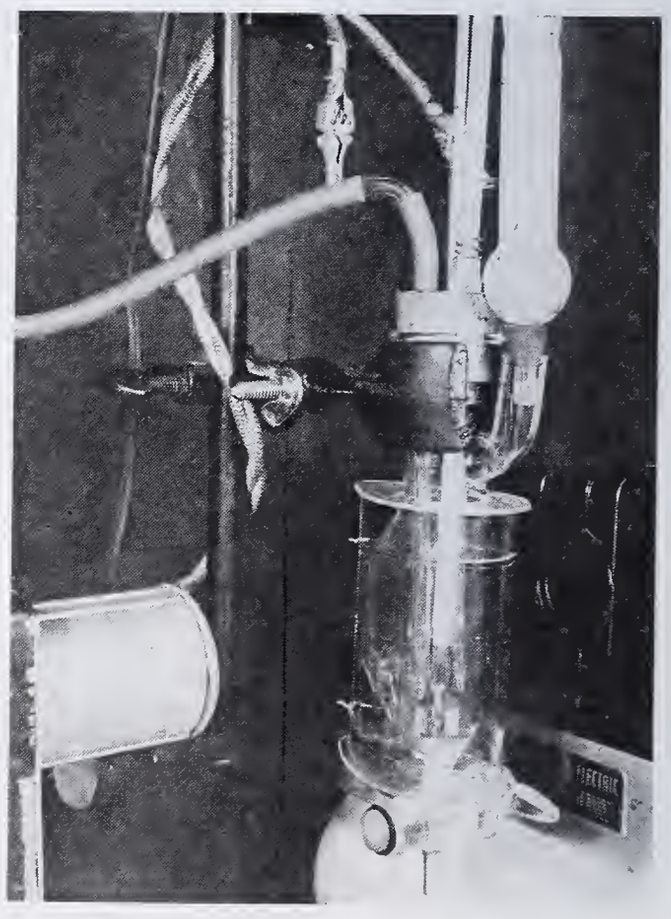

Figure 4. Light Source and Photocell

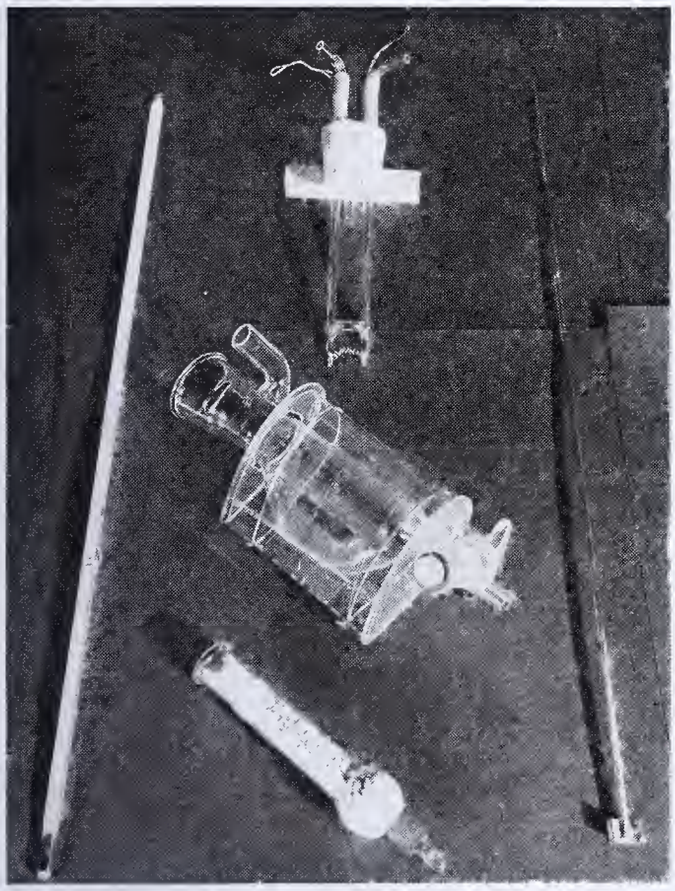

Figure 5. Sample Container Disassembled

Tables I and II show the reproducibility with the apparatus and the close agreement with results obtained by the A.S.T.M. procedure.

Table III covers a few tests which indicate the adaptability of this apparatus to dark petroleum products. The samples cover the A.S.T.M. color range from −7 to very much darker than 8. Incidentally, while there is no doubt a limit of sample color beyond which this apparatus may not function, changes in sample container design and a more sensitive light source (possibly infrared) and photocell combination may permit the testing of practically all dark products.

Table III. Aniline Point of Dark-Colored Petroleum Products

No.	Sample Color, Lovibond (500 Amber Series), 0.25-Inch Cell: As is	Sample Color, Lovibond (500 Amber Series), 0.25-Inch Cell: Diluted with kerosene 9 to 1	A.S.T.M.	Results Proposed Automatic, ° C.
A	350	...	7−	82.2
B	450	...	8−	116.8
C	700	...	8+	95.5
D	>750	95	8+	104.4
E	>750	>750	8+	81.3

Figure 2 shows the general setup.

Figure 3 pictures the sample container plus jacket, etc., and Figure 4 pictures container in position between light source and photocell. The clip which prevents the thermometer from slipping through the glass sleeve in the stopper is replaced by a tight-fitting rubber sleeve when testing samples whose aniline points are below room temperature.

Figure 5 pictures sample container, U-tube, stirrer, and thermometer disassembled.

Tentative steps are under way for the manufacture of this apparatus by the Precision Scientific Company, Chicago, Ill.

ACKNOWLEDGMENT

The author wishes to extend his appreciation to J. L. Koehler and A. Herzog for determining the A.S.T.M. aniline points, to J. H. Lange for obtaining the electric-eye apparatus, and to R. Work for making the glass apparatus.

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An Automatic-Recording Ultraviolet Photometer for Laboratory and Field Use

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The rates of adsorption processes or chemical reactions involving a gas which absorbs ultraviolet light can be followed readily with an ultraviolet photometer. An automatic-recording instrument is described capable of detecting concentrations of the order of a part per million. This apparatus has been applied to the evaluation of the efficiency of adsorbents against toxic gases, to field studies of the travel of gas clouds, and to fundamental investigations in the kinetics of gaseous reactions.

IN TESTS of the protection of absorbents against toxic gases and in field studies of the travel of gas clouds, it is highly desirable to have an instrument which will follow rapid changes in concentration and will detect minute quantities of certain test gases. In laboratory tests of the efficiency of adsorbents, a knowledge of the variation of effluent concentration with time is necessary for both practical and theoretical assessment, and since this concentration-time curve (Figure 1) is frequently very steep, a rapid, recording analyzer is most desirable. In field tests a cloud of toxic gas may pass a station in less than a minute and may exhibit large variations in concentration within that short period (Figure 2), so that a rapid-recording device becomes essential.

A number of the important war gases—for example, phosgene (*2*) and chloropicrin (*4*)—exhibit strong absorption of ultraviolet radiation. This property may be used to advantage as an analytical tool and has been so applied by Woodson (*9*) and others (*1*, *3*, *6*). The instrument described in this paper has a number of advantages over those previously described. For increased precision in analysis, a null-point measuring circuit has been introduced similar to one already described (*3*). To avoid the neces-

sity of frequent recalibration as the intensity of the light declines or when the source must be replaced, an arrangement has been inserted whereby the absolute intensity of the radiation may be measured. Finally, provision has been made for the use of an

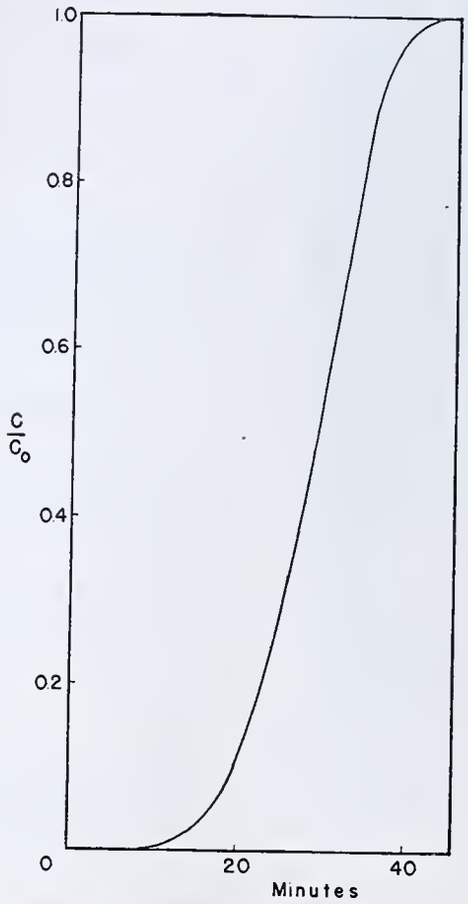

Figure 1. Effluent Concentration from a Column of Charcoal

automatic-recording microammeter which may be introduced or removed from the circuit by the flip of a single switch.

PRINCIPLE OF THE PHOTOMETER

In quantitative photometric work it is customary to treat the data in terms of Beer's law

$$\log(I_0/I) = e\,c\,d \tag{1}$$

where I_0 is the intensity of the light passing through an absorption cell of d cm. length, containing none of the light-absorbing material, I is the intensity of the light traversing the same cell containing the light-absorbing substance at a concentration of c moles per liter, and e, the molecular extinction coefficient, is a quantity which depends on the compound, on the wave length, and occasionally on the concentration. In the present instrument, the light intensity is translated into a voltage. Since the number of electrons emitted by the photocell is proportional to the intensity of the incident light and since the voltage, V, is created by the flow of electrons through a constant resistance, R_1, it follows that

$$V = kIR_1 \tag{2}$$

where k is a constant.

Consequently Beer's law assumes the form

$$\log \frac{V_0}{V} = e\,c\,d \tag{3}$$

To obtain increased sensitivity in detection, balanced-photocell, electrical circuits have been devised to read the change from V_0 when a light-absorbing substance is present. This change, v, is related to V by the equation

$$v = V_0 - V \tag{4}$$

Consequently, Beer's law assumes the form

$$\log \frac{V_0}{V_0 - v} = e\,c\,d \tag{5}$$

A schematic diagram of the optical and electrical system is illustrated in Figure 3. The principle of its operation is simple.

If light is allowed to fall on the photocell, P_1, a stream of electrons will flow toward and through the grid leak, R_1, and will create a potential drop across this high resistance. If S_1 is switched to the left-hand circuit, but S_6, S_7, and *POT.* are all adjusted to zero e.m.f., then the potential drop across the grid leak will be impressed on the amplifier and cause a deflection in the plate-current galvanometer, *GALV.*, or on the recorder. The magnitude of this potential drop can be measured accurately by imposing a counterpotential through the batteries, B_2 and B_3, and the potentiometer, *POT.*, until the galvanometer, or recorder, returns to its initial reading. In this manner the value of V_0 may be determined.

If now two beams of light of equal intensity are allowed to fall on the two photocells, P_1 and P_2, respectively, and if these photo-

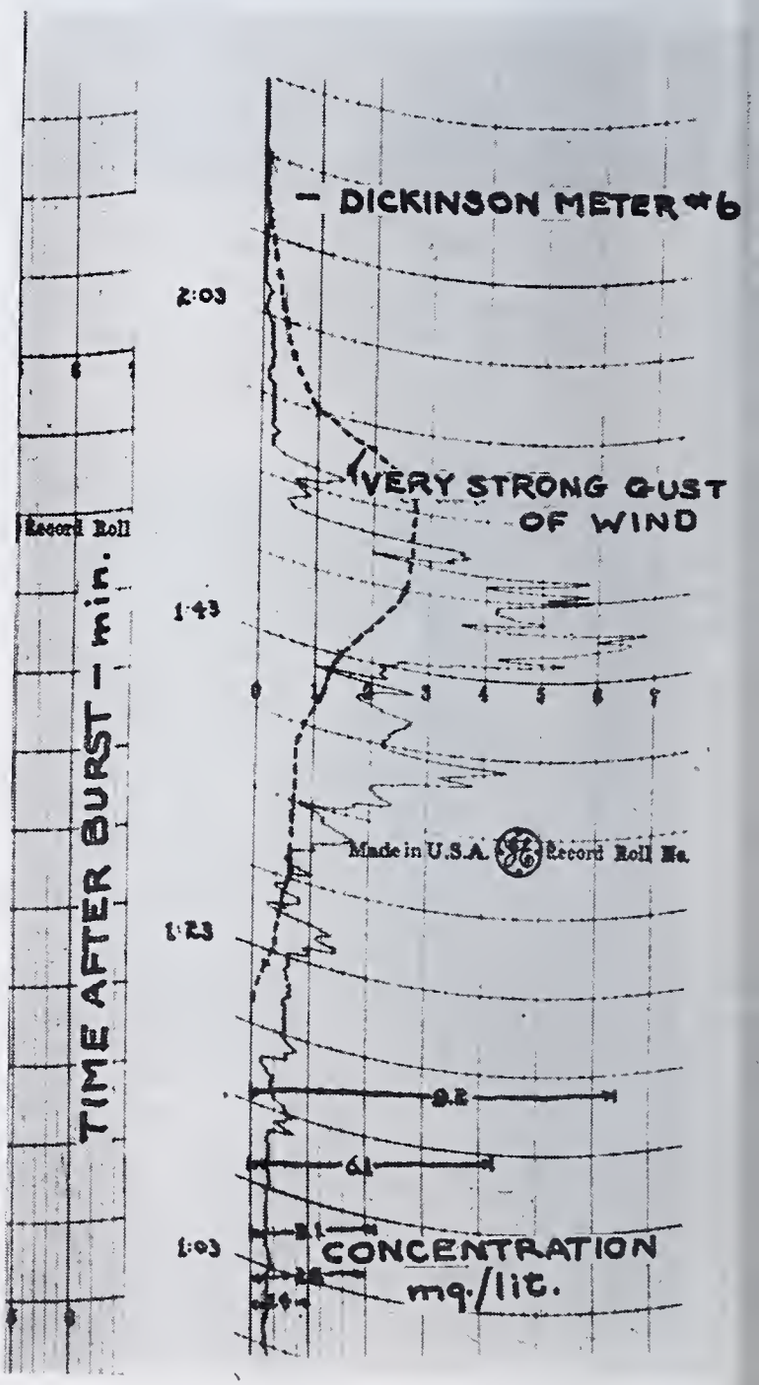

Figure 2. Automatic Record of Gas Concentration in a Field Test

cells are identical in response, no net flow of electrons through R_1, and consequently no potential drop, is observed. (In practice, of course, the photocells are not identical, and it is necessary to use a set of diaphragms to balance the outputs, as is described in the section on operation.) If a light-absorbing substance is interposed between the light source and one of the photocells, the electrical balance is disturbed and a potential drop will be impressed across R_1. This potential may be balanced, with S_1 switched to the left, by potentiometer, *POT.*, and batteries B_2 and B_3 if necessary, to obtain the value of v. To detect very small changes in v, S_1 is switched to the right and a Leeds & Northrup student-type potentiometer introduced into the circuit. A difference of about 0.001% in light intensity can be detected thereby.

When the automatic recorder is to be used, S_3 is switched to the right, and the changes in intensity of light, due to changes in concentration of absorbing material, are continuously recorded on a chart. The deflections are then calibrated by imposing a counter-potential on the grid leak by means of the potentiometer and batteries B_2 and B_3.

CONSTRUCTION OF PHOTOMETER

Light Source. The most recent model of the photometer has been constructed to enable the use of either a General Electric T-10 germicidal lamp or a Westinghouse WL-793 sterilamp as the source of ultraviolet light. The GE lamp has proved more satisfactory but for a time was not manufactured. Whereas 95% of the radiation of the GE lamp is at 2537 Å. (*5*), 84% of the radiation of the Westinghouse lamp is concentrated at this wave length (*8*).

The current going through the GE lamp was stabilized by a constant-current circuit whose theory has been discussed by Steinmetz (*7*) and which has been used for the same purpose by Hanson (*1*). Constant voltage is obtained with the aid of a Sola transformer or a Raytheon voltage stabilizer. With the WL-793 lamp it is also necessary to use a special 220-volt transformer.

Provision has been made also for operating the GE lamp from a direct current source. For this purpose three 45-volt B batteries (Burgess No. 21308) are connected in series with a 2500-ohm, 50-watt rheostat (Ohmite No. 0328), and inserted into the light-source circuit in place of the 110-volt alternating current. To start the lamp, the rheostat is turned to zero resistance, for only with the full 135 volts across the electrodes will the mercury arc strike. Once the lamp is in operation, however, the rheostat is adjusted to decrease the intensity of the ultraviolet radiation to a suitable value. Under normal operating conditions, the direct current flowing through the lamp is about 20 milliamperes.

Absorption Cell. Side arms were blown on to a Pyrex tube, which was then cut with the aid of a glass saw to a length of 20 cm. Windows of Corning ultraviolet-transmitting glass No. 791 were

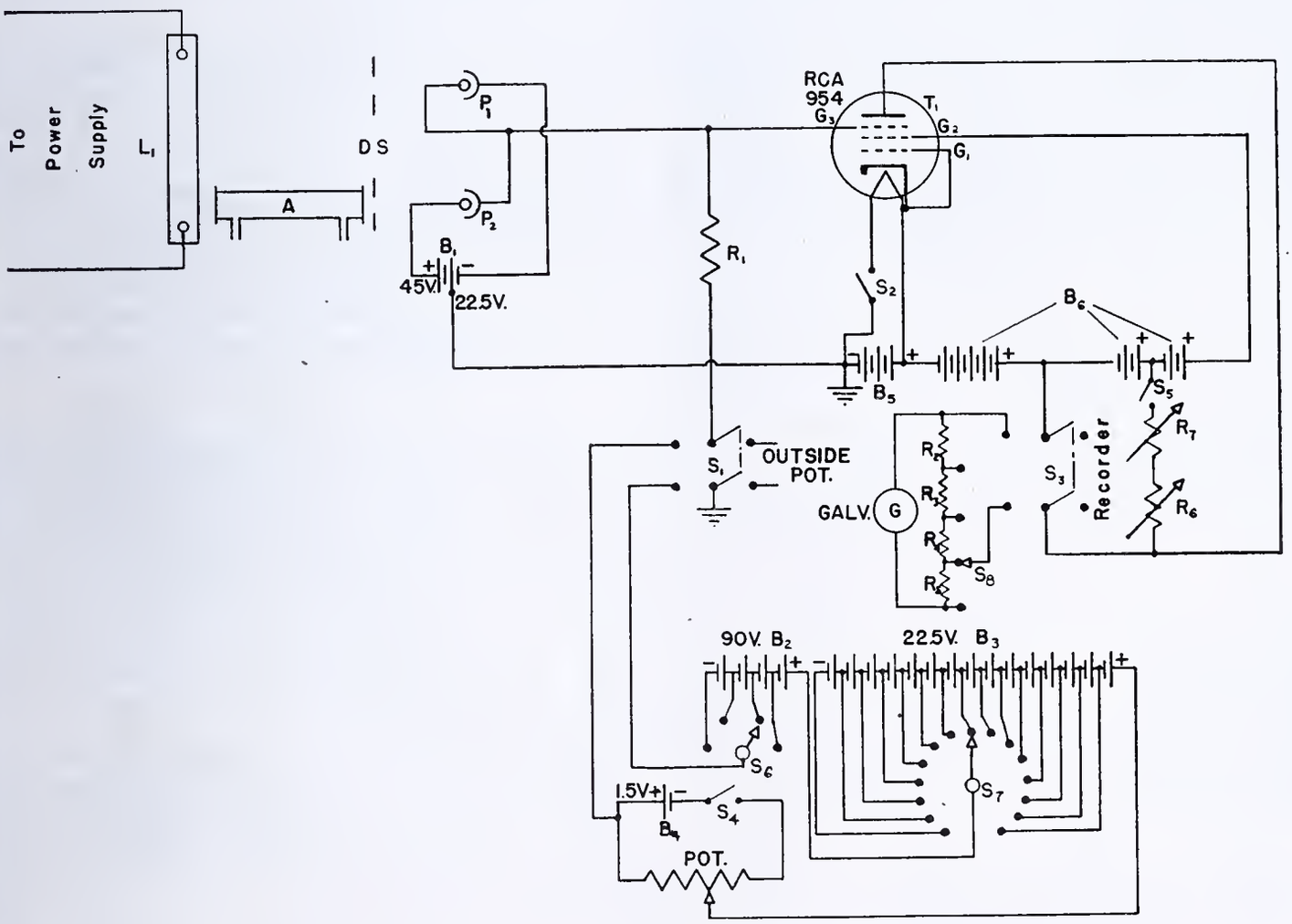

Figure 3. Schematic Diagram of Ultraviolet Photometer

Galv. GE galvanometer 32 C 205 G 1
P_1, P_2. R.C.A. 935 phototubes
R_1. I.R.C. type H 1 2000 megohms
R_2. 10 ohms
R_3. 200 ohms
R_4. 1000 ohms
R_5. 10,000 ohms
R_6. General Radio Co. potentiometer type 371-A, 25 watts, 1000 ohms
R_7. General Radio Co. potentiometer type 371-A, 25 watts 50,000 ohms
Pot. General Radio Co. potentiometer type 314-A, 6 watts, 20,000 ohms
T_1. RCA 954
S_1. Double-pole double-throw toggle switch
S_2, S_4, S_5. Single-pole single-throw toggle switch
S_3. Double-pole double-throw jack switch, Mallory 762
S_6, S_8. Single-pole 5 place Mallory selector switch 3215-J
S_7. Single-pole 17 place Mallory selector switch 32117-J
B_1. Burgess W30 BP
B_2. 2 Burgess 5308 in series
B_3. 3 Burgess 5540 in series
B_4. Burgess 4 F H
B_5. 3 Burgess 4 F H in series
B_6. Burgess 5156 with Fahnstock clips
L_1. G E T-10 germicidal lamp or Westinghouse WL-793 sterilamp
DS. Diaphragm and shutter
A. Absorption cell
110-volt A.C. obtained from constant voltage source connected to building mains

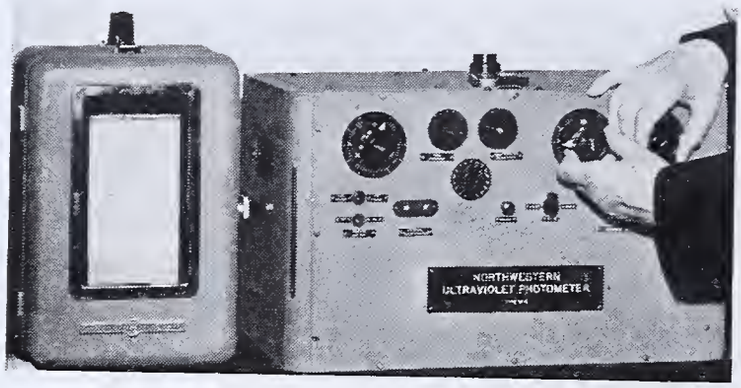

Figure 4. Ultraviolet Photometer and Recorder

sealed to the tubes with Cenco Sealstix cement. Glass tubes were then sealed to the side arms and led outside the photometer cabinet, so that they served as an inlet and outlet for the gas to be analyzed.

Detecting and Recording Circuits. RCA 935 phototubes serve as the light-sensitive elements. The photocurrents are amplified in an electrometer circuit using an RCA 954 acorn pentode tube as previously described (*3*). The details are specified adequately in the legend attached to Figure 3.

The automatic recorder is a General Electric recording microammeter, type 8CE1CK12, with a full-scale deflection corresponding to 56 microamperes. A suitable Ayrton shunt has been introduced to decrease this sensitivity for use in cases where high concentrations and hence large currents are encountered.

For use with an alternating current source, the entire apparatus, with the exception of the automatic recorder, was assembled compactly in a single metal cabinet of 20 × 12 × 12 inches. The complete assembly and recorder are illustrated in Figure 4. The cost of a complete instrument, including recorder, is about $800.

OPERATION OF PHOTOMETER

The first step in operating the photometer is to start the electrical current flowing through the ultraviolet lamp and through the amplifying circuit. There is always a drift while the electrometer tube, ultraviolet lamp, and constant-voltage transformer are warming up. A preliminary period of about half an hour is necessary before the galvanometer deflection, a measure of overall stability, becomes sufficiently steady for measurements to be made. During this period the shutters are kept closed.

The electrical circuits having been stabilized, one proceeds to measure V_0. The shutter in front of P_1 is opened and the diaphragm and counterpotential are adjusted to a suitable balance. The balance may be obtained with the galvanometer, *GALV.*, or the recorder as the null-indicating device. The exact value of the counterpotential is then recorded.

The counterpotential is then decreased to zero and the shutter in front of P_2 is opened. The diaphragm of this compartment is adjusted so that the light intensities impinging on the photocells are equalized, as indicated by a null reading in the plate-current galvanometer, the sensitivity of the shunt being adjusted progressively to its highest value.

During the preceding operations pure dry air is maintained in the absorption cell. Once the instrument has been balanced, one may introduce the absorbing gas. Its presence decreases the intensity of the light reaching one of the photocells, and creates an unbalance which may be counteracted by applying a potential with *POT.*, B_2 and B_3. The magnitude of this potential is v. With the aid of Equation 5 and a calibration curve the concentration may be determined readily.

The batteries in the photometer will run down slowly. The current drain on B_5 is high and these dry cells must be replaced every three or four months, if the instrument is operated daily. The drain on B_4 and B_6 is small and that on B_1, B_2, and B_3 extremely small, since, in the latter case, practically no current is drawn in its very high-resistance circuit. All these batteries are replaced annually. For highest precision, those in the grid-leak circuit should be calibrated from time to time, by means of a potential divider and a potentiometer, since their voltages are used in the calculations. As the voltages appear in a ratio in Equation 5, however, errors due to the running-down of these dry cells are small.

TECHNIQUE OF GAS ANALYSIS

Various arrangements have been used to introduce the gas to be analyzed into the absorption chamber. In laboratory work, the gas-testing line is usually operated under a pressure of a few inches of water, and the gas effluent from the absorbent may be led into the absorption cell by suitable leads of glass tubing. Standard-taper joints should be incorporated into this line, for appreciable erorrs are introduced by the presence of rubber connections when organic vapors are being analyzed.

In field work it is convenient to attach a small pump to the exit end of the absorption cell and thereby suck the surrounding gas through the absorption cell. A three-way stopcock and a tube of charcoal and drying agent are attached to the entrance end of the absorption cell, so that pure dry air may be introduced into the system during the initial adjustment of the instrument.

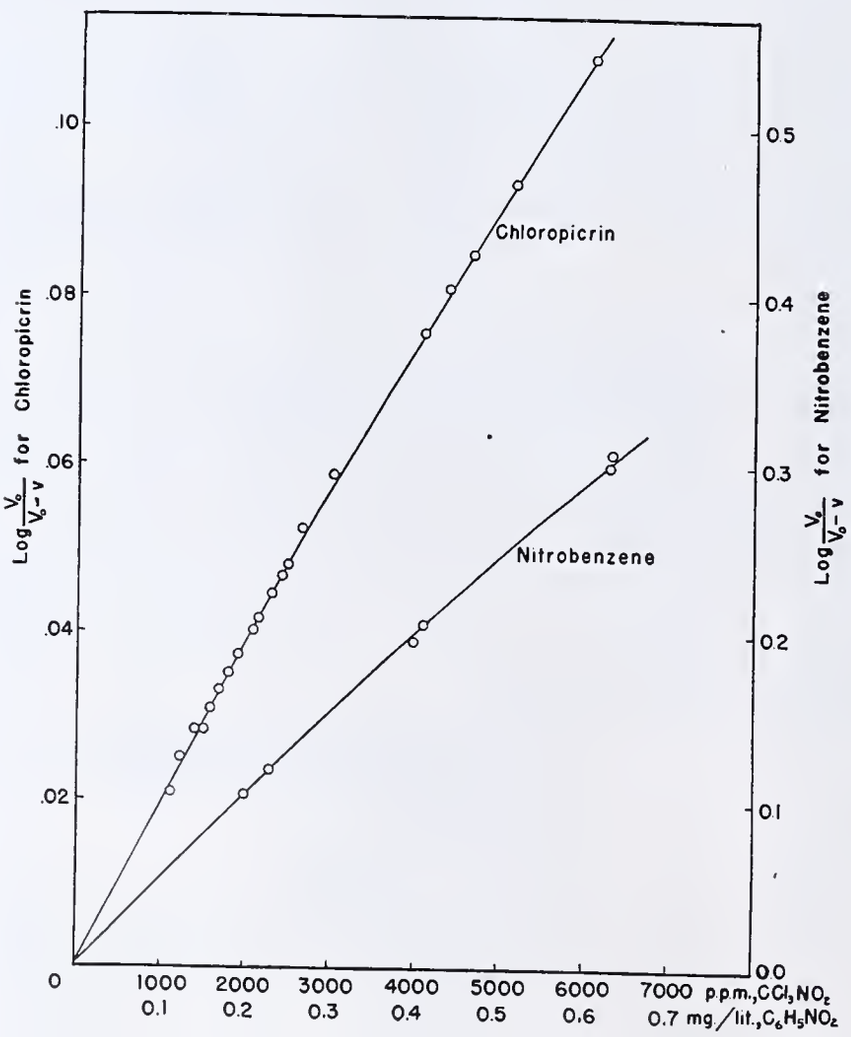

Figure 5. Calibration Curves for Chloropicrin and Nitrobenzene

Table I. Sensitivity of Detection

Gas	Sensitivity *P.p.m. (by volume)*
Chloropicrin	1
Phosgene	1
Mustard gas	3
Chlorine	3
Nickel carbonyl	0.5
Iodine	0.5
Toluene	0.2
Chloroacetophenone	0.03
Mercury	0.0001

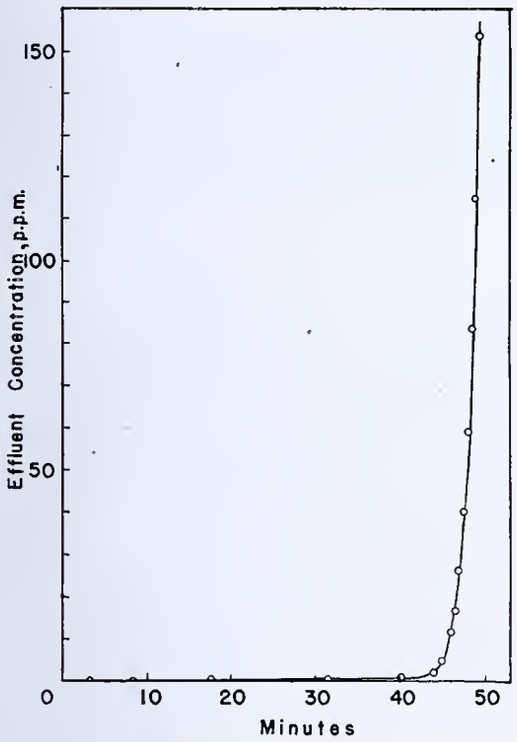

Figure 6. Penetration Curve at Low Effluent Concentrations

In many field and house trials it was necessary to maintain a large number of sampling stations, so that the duration of presence and concentration of the toxic gas could be evaluated at various distances from the point of release. The construction of photometers for each one of these stations would have been unduly expensive and time-consuming. Therefore, instead of a photometer, a rack of a dozen or more sampling bulbs was set up at each station.

These bulbs consisted of a long body of about 50-mm. Pyrex tubing, rounded at one end and sealed at the other to standard 10-mm. glass tubing. The bulbs were evacuated prior to the release of the gas, and then sealed off. At specified intervals during a "shoot", the tapered tip of a bulb was scratched with a file and then broken. Air containing the toxic gas rushed into the bulb until the pressure inside was equal to that outside. The tip was then sealed with a suitable wax and the bulb was returned to the rack for subsequent analysis. At the conclusion of the "shoot" the bulbs were all assembled at a central station containing one or two photometers and the contents of each were analyzed. For analyses in these cases, the sampling bulb was attached to one end of the inlet three-way stopcock of the absorption cell, which was then evacuated up to the stopcock, and the latter was then opened, so that the contents of the bulb could diffuse into the absorption cell.

APPLICATIONS

In all analyses a calibration curve must be obtained first. Typical curves, for chloropicrin and nitrobenzene, are shown in Figure 5. These were obtained by passing each gas at a series of known concentrations through the photometer and recording the voltages, v, that were observed. The function indicated by Equation 5 was then plotted, as is illustrated in Figure 5. The curves obtained are not linear, probably because of the stray light in the radiation source. The nonlinearity introduces no difficulty however, for in any test run concentrations may be determined readily by reference to a large-scale reproduction of Figure 5.

In a theoretical analysis of the nature of the removal process in a flowing system, it is necessary to have a detailed time-history of the concentration effluent from the absorbent. A typical curve of this type is shown in Figure 1. With manual control it is possible to obtain a reading every 30 seconds. With the recorder one obtains, of course, a continuous record. In either event the concentrations are essentially instantaneous values, the lag in the electrical circuits being completely negligible.

The extreme sensitivity of detection is illustrated in Figure 6, which shows a penetration curve for chloropicrin passing through charcoal. Individual readings, collected by manual control, are shown in this figure. This curve makes it possible to study the nature of the penetration at very low effluent concentrations.

A typical field test, as well as an example of the use of the recorder, is illustrated in Figure 2. For purposes of comparison the readings of an automatic-recording conductivity meter are shown on the same graph (scale different). It is obvious that the photometer retains the "fine-structure" of the variation in gas concentration, whereas the conductivity meter smooths out relatively large variations. As is also illustrated in Figure 2, the dense portion of the gas cloud passed the station at which the photometer was situated in a fraction of a minute, yet details of the variations in concentration have been recorded clearly.

The instrument is also applicable to many kinetic problems. It has been used by Blacet, Blaedel, and Lewis, in this laboratory, to study the rates of conversion of nitric oxide into nitrogen dioxide. Their work will be described in a subsequent publication.

In general the photometer is applicable to any kinetic or thermodynamic problem requiring the analysis of a gas which absorbs ultraviolet radiation. Included in this category are organic conjugated compounds as well as ketones, aldehydes, and nitro derivatives. A few typical sensitivities which have been determined with the present instrument are shown in Table I.

There are, of course, many compounds which do not absorb ultraviolet radiation of 2537 Å wave length and for these substances the instrument is not suitable. A few of the war gases in this latter category are arsine, hydrogen cyanide, and cyanogen chloride. Where the instrument is applicable, however, it offers a very rapid and convenient method of analysis.

ACKNOWLEDGMENT

The authors are indebted to William E. Roake, L. P. Saxer, Charles Eckert, and Frank Lamb for their aid in assembling and testing a number of photometers. The advice and assistance of Norman Nachtrieb in the design and construction of some of the critical parts of the instrument are also gratefully acknowledged.

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Quantitative Analysis of Isomeric Cresols and Cresol-Phenol Mixtures

By Ultraviolet Absorption Spectra of Vapors

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A procedure for the quantitative analysis of isomeric cresols and cresol-phenol mixtures is described. Spectrograms of the vapor in equilibrium with liquid mixtures are taken under fixed conditions for a number of synthetic samples. Working curves are prepared from the measured densities of select bands in the absorption spectra and the known concentrations in the samples. The mean deviation between the composition of the synthetic isomeric cresol samples and the composition of these samples as obtained from the working curves is less than 2%. Phenol can be detected in cresol mixtures when present in amounts as low as 0.3% and can be analyzed for at this and higher concentrations with about the same precision as that mentioned above.

THE ultraviolet absorption spectra of aromatic molecules in the vapor state have a number of features which suggest their use in analytical chemistry. All aromatic compounds absorb light in the region of 2500 to 3000 Å., owing to an electronic transition involving the excitation of the pi electrons in the ring, corresponding to the A_{1g}–B_{2u} transition in benzene (*4*, *5*).

The electronic transition is very structure-sensitive and the ultraviolet absorption spectra should be effective in bringing out peculiarities in compounds of similar structure.

The ultraviolet absorption of aromatics in the liquid state has been widely used in analyses. The use of the liquid state has the important disadvantage that the fine structure so characteristic of the vapor spectrum is largely wiped out and with it much of the individuality of the spectra of the various aromatic compounds. Cole (*2*) has analyzed air for toluene and benzene, using ultraviolet absorption. In 1944 Berton (*1*) discussed the analysis of aromatic compound mixtures by means of vapor ultraviolet spectrography. He reproduced spectrograms of mixtures of aromatic compounds but gave no quantitative data except the results of analyses for toluene and benzene in air. In this he had been completely anticipated by Cole who, three years earlier, published complete analytical data, including the working curves for both toluene and benzene. Berton probably did not have access to the American journals during the war.

EXPERIMENTAL

The spectrograph used in this work and described in earlier investigations (*3*) was a 3-meter grating instrument with modified Eagle mounting. The absorption cell was quartz, 70 cm. long, with two outlets, one to a reservoir holding the sample, the other to a Hyvac pump through a stopcock and trap. Ground-glass joints lubricated with Apiezon L were used. A 2.5-kv.-amp. hydrogen discharge tube provided the illumination.

Synthetic samples were prepared and introduced into the reservoir and were maintained at 25° C. The cell, cleaned by rinsing with acetone and ether and with the ground-glass joints completely relubricated after the preceding run, was exhausted for 10 minutes and a time interval of 10 minutes was allowed for attaining equilibrium after the system was isolated from the pump. All the exposures were taken in the first order of the spectrograph (slit width, 0.05 mm.) and were of 10 minutes' duration. Eastman 103-0 spectrographic plates were used and given a tray development of 8 minutes with D76c at 19° C. with manual agitation, this development being estimated to give a gamma of about 1 (a degree of contrast such that the slope of the curve of density versus the logarithm of the exposure would be about 1). The developer used had to be mixed very carefully if consistent results were to be obtained from different batches of developer. The plates were scanned with a Leeds & Northrup microphotometer.

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CRESOL MIXTURES

PREPARATION OF WORKING CURVES. Spectrograms were taken of the three pure cresols and of a number of synthetic mixtures. In Figure 1, reproduced as negative prints, are the spectra of the pure components. In Figure 2 are the microphotometer tracings of these plates, as well as of the plate obtained from sample 5 of Table I. The scale on the left of each microphotometer tracing is a density scale obtained by running an Eastman stepwedge with each plate for the purpose of calibrating the microphotometer. This was necessary, since the sensitivity of the microphotometer was changed from plate to plate in such a manner as to bring out as much detail as possible.

The strongest bands of the spectra of *o*-, *m*-, and *p*-cresol are designated, respectively, as A_0, A_m, and A_p with wave lengths 2744, 2779, and 2830 Å., respectively. It will be evident from an examination of the spectrograms of Figure 1 or the tracings of Figure 2 that the A_0 band and the A_p band are relatively independent. Thus the density of the center of each band will serve as an independent measure of the concentration of the corresponding cresol in the sample. In Figures 3 and 4 are given the working curves for *o*-cresol and *p*-cresol as they were obtained from the various synthetic mixtures. The "density Δ" is the den-

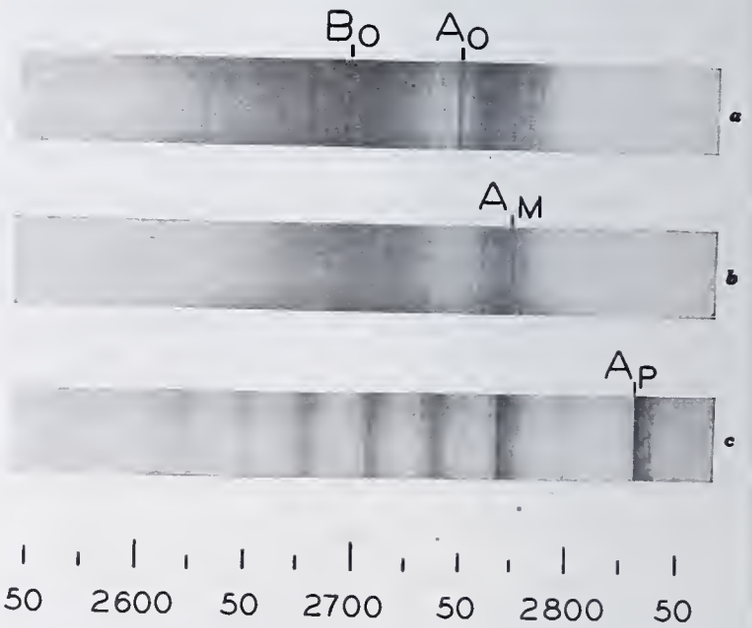


Figure 1. Near Ultraviolet Absorption Spectrograms of Cresol Vapors

a, *o*-Cresol. *b*, *m*-Cresol. *c*, *p*-Cresol.

ity difference between the background of the band and the cen-er of the band. This roughly cancels the variations in the in-ensity of the light source and in development.

The meta band, A_m, has a relatively low extinction coefficient nd is overlapped by an ortho band of almost equal intensity. Therefore it was not possible to determine the meta concentra-ion by the direct method which was used for the ortho and para somers. The difference in density between bands from two different compounds in a single mixture is a more sensitive func-ion of concentration than the density of either band alone. The lensity difference, called Δ', was taken between band A_m and he second strongest ortho band, labeled B_0 (λ = 2694 Å.) on the pectrograms of Figure 1. B_0 was used instead of A_0 to avoid he effect of strong absorption mentioned below. Since the para somer also absorbs slightly in this region, a working curve of Δ' versus per cent meta was first prepared from meta-ortho mix-ures. The three-component synthetic mixtures were then nalyzed and a correction curve was determined (see Figure 5) hat would eliminate the effect of the *p*-cresol on the two-compo-nent density Δ'–% meta curve mentioned previously. The pro-cedure is as follows:

The amounts of ortho and para are first determined. Then the para density correction is determined from Figure 5 and added to the Δ', determined from the microphotometer record. The composition of meta in terms of ortho is then read off Figure 6, using the corrected density, Δ'. Then meta is known in terms of ortho and the per cent meta in the complete sample may be calculated.

Each working curve that is drawn represents an average of the results in terms of the density Δ as a function of concentration.

Discussion of Results. The Beer-Lambert law is fairly well obeyed for low concentrations of the components but not for the higher concentrations, particularly in the case of the ortho isomer. This is because the absorption of these bands is so strong that the exposure in the center of the bands falls below the linear portions of the density-exposure curve given in Figure 6. (This curve is the experimentally determined characteristic

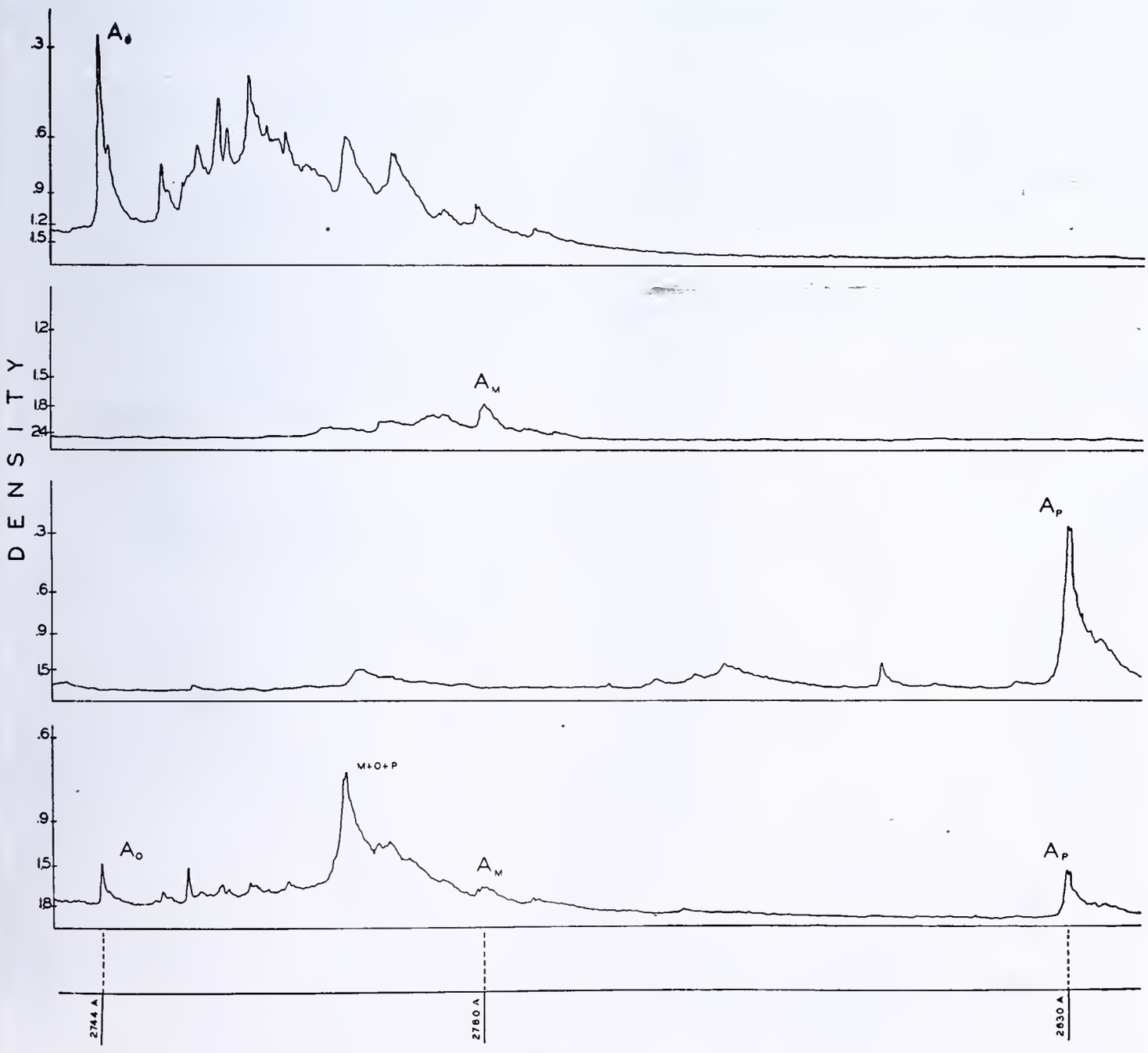


Figure 2. Microphotometer Tracings of Spectrograms of Figure 1

a. *o*-Cresol. *b*. *m*-Cresol. *c*. *p*-Cresol. *d*. Sample of 30.1% ortho, 31.7% meta, and 38.2% para

curve of Eastman 103-0 spectrographic plates when processed as described above.) Consequently, samples of 50% or higher ortho or 70% or higher para cannot be analyzed directly. However, a known amount of para or ortho, respectively, or of meta may be added to bring the relative concentrations down. Another possible method of analyzing for high percentages of ortho or para involves the use of an additional short cell, whose length relative to the length of the original cell is known.

The straight-line portion of the curve indicates also that Raoult's law is obeyed, at least over that region.

In Table I are given the concentrations of the three cresol isomers in various synthetic samples as determined from the working curves and the experimental data, except for the samples with very high ortho or para concentrations. The compositions of the corresponding synthetic samples are given in the same table. The mean deviation between the two is less than 2%.

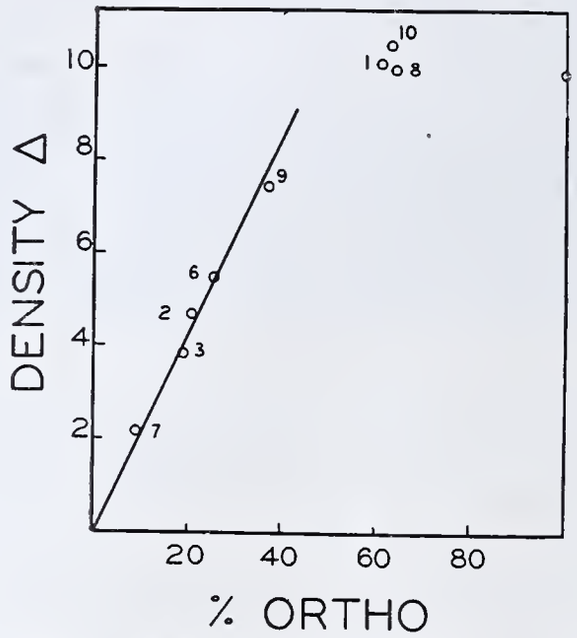


Figure 3. Working Curve for o-Cresol Based upon Band A_0 of Wave Length 2744 Å.

Δ is density difference between background and center of band

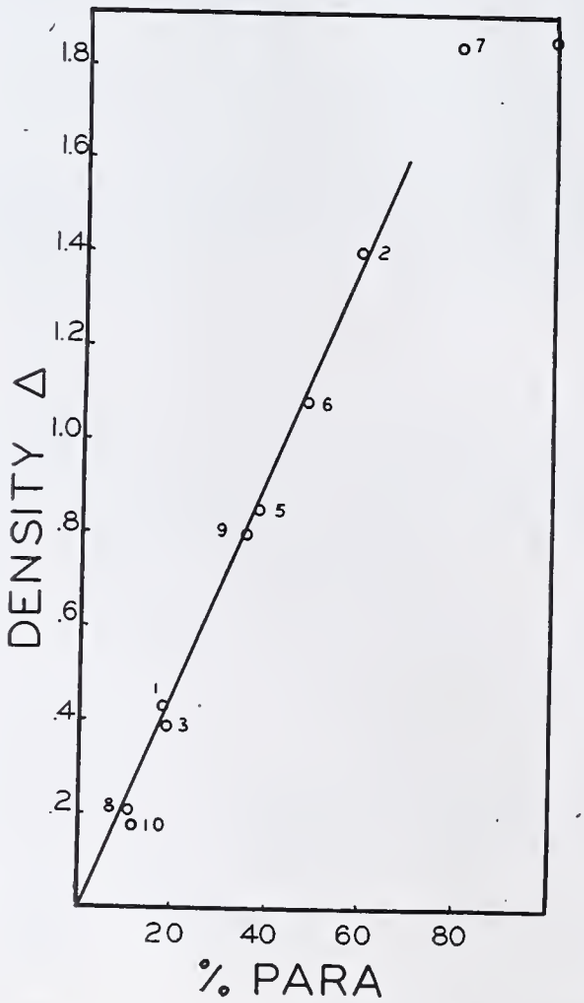


Figure 4. Working Curve for *p*-Cresol Based upon Band A_p of Wave Length 2830 Å.

CRESOL-PHENOL MIXTURES

Preparation of Working Curves. In Figure 8 are reproductions of spectrograms of pure phenol and of a phenol-cresol mixture of 16.4% phenol. Figure 9 shows microphotometer tracings of the above spectrograms. It will be noticed from 9, *b*, that the spectrum of phenol is much more strongly developed than that of the *o*-, *m*-, or *p*-cresols. The strong bands (A_0 and A_p) of the *o*-cresol and *p*-cresol spectra are, however, completely independent of the phenol spectrum, whereas the relatively weak spectrum of *m*-cresol is completely lost in that of phenol.

The band marked *P* of wave length 2636.7 Å. in the phenol spectrum was chosen for the analysis because of its moderate

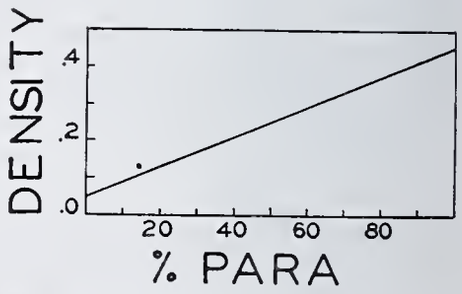
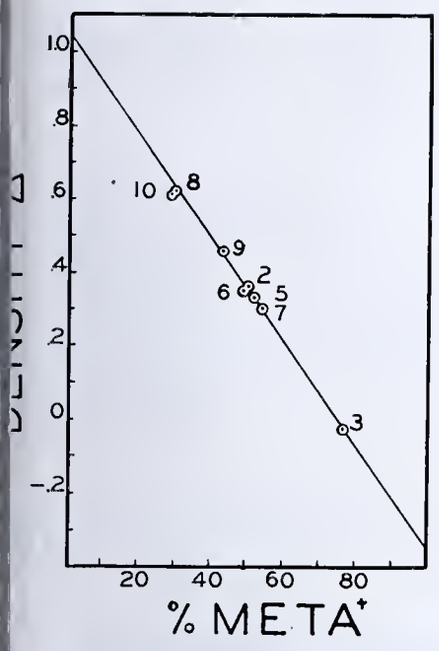


Figure 5. Correction Curve Used in Meta Analysis

Density corresponding to determined percentage of para is to be added to density difference, $A_m - B_0$

Table I. Composition of Samples

Sample No.	Synthetic Sample		Determined from Working Curve
1	Ortho	61.4	..
	Meta	20.7	..
	Para	17.8	20
2	Ortho	20.6	22
	Meta	20.0	21
	Para	59.4	61
3	Ortho	19.4	18
	Meta	61.8	59
	Para	18.8	17
5	Ortho	30.1	29
	Meta	31.7	30
	Para	38.2	37
6	Ortho	25.7	26
	Meta	26.3	26
	Para	48.0	47
7	Ortho	9.4	10
	Meta	10.4	12
	Para	80.2	80
8	Ortho	64.2	..
	Meta	25.1	..
	Para	10.7	10
9	Ortho	37.3	35
	Meta	27.4	30
	Para	35.3	35
10	Ortho	63.1	..
	Meta	26.2	..
	Para	10.6	9



rrected density difference between A_m of λ = 79 Å. and B_0 of λ = 2694 Å. is plotted against rcentage of meta, where meta + ortho content is taken as 100%

Figure 6. Working Curve for *m*-Cresol

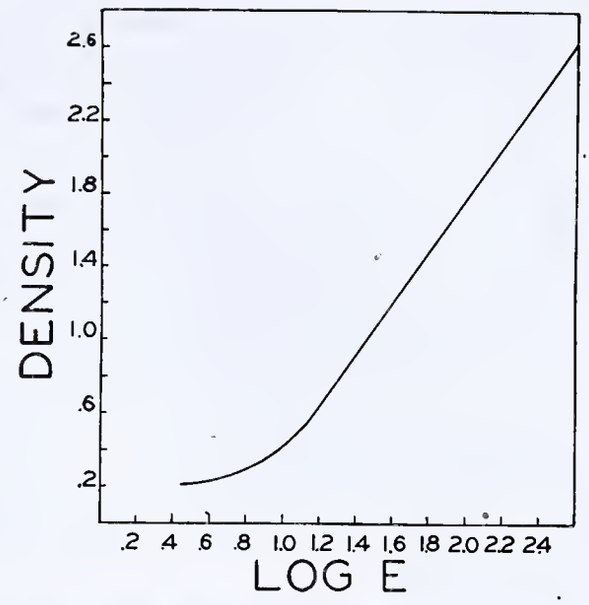


Figure 7. Characteristic Curve of Eastman 103-0 Spectrographic Plate, Developed as Described in Text

ensity, its complete independence of the cresol spectra, and sharpness. In these mixtures, then, *o*-cresol, *p*-cresol, and enol can be determined directly, and *m*-cresol is known if it is only remaining component of the mixture.

The apparently small differences in the intensities of the strong enol bands shown in the tracings in Figure 9 are due to the t that the exposures in these bands fall on the rather flat, insitive toe of the characteristic curve of the photographic ulsion (see Figure 7).

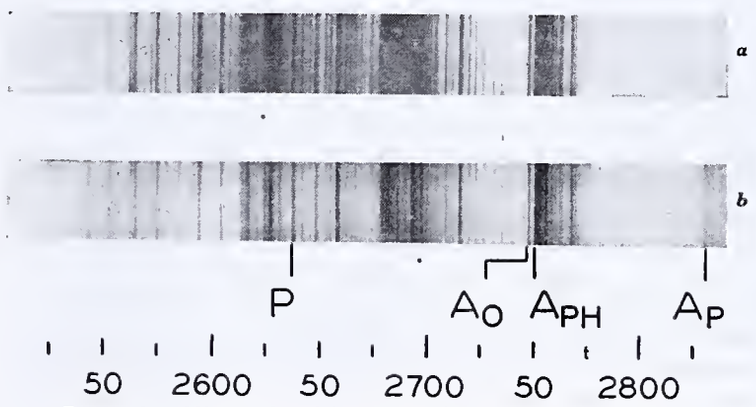


Figure 8. Ultraviolet Absorption Spectrograms of Vapors

a. Pure phenol. *b*. Sample 4 of phenol-cresol mixtures

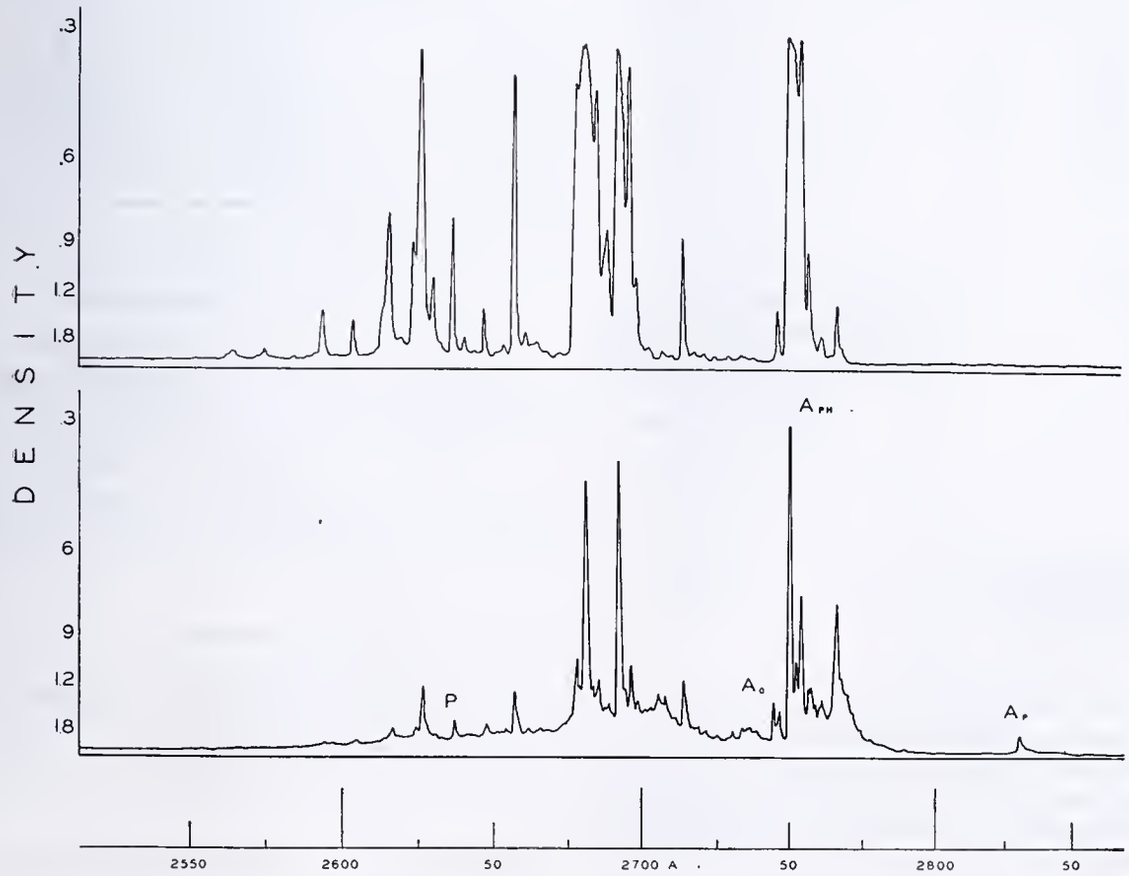


Figure 9. Microphotometer Tracings of Spectrograms of Figure 8

In Figure 10 is given the working curve for phenol. The density Δ that is plotted against the phenol concentration is the difference in density between the center of the band *P* and the region immediately outside the sharp edge of the band.

RESULTS OF ANALYSIS. The curve in Figure 10 departs from linearity at concentrations of phenol greater than about 40%. This departure is due apparently to an unsymmetric development of background absorption and not to the lack of linearity in the response of the photographic emulsion at the densities involved. Samples containing more than 40% of phenol can be analyzed by following the suggestions given above.

A comparison is given in Table II between the percentages of phenol in the synthetic samples and those read from the working curve. There is a mean deviation between the two of less than 3%.

p-Cresol and *o*-cresol were determined in the first four samples taken in order to check the validity of the cresol working curves in a phenol-cresol system. The results given in Table III indicate that the phenol has not affected the *o*- and *p*-cresol bands used in the analyses.

ANALYSIS OF SAMPLES OF LOW PHENOL CONCENTRATION

A working curve for low concentrations of phenol is given in Figure 11. The strong band of phenol marked A_{PH} (λ = 2750.3 Å.) in Figure 9, *b*, was used for this curve. As a weak ortho band falls at the same wave length, a slight correction for the percentage of *o*-cresol in the sample must be made. This correction was determined from separate samples containing no phenol, but was too small to justify a complete eorrection curve and can be taken as a density of 0.03 to be subtracted from the phenol density difference for concentrations of *o*-cresol up to 20%, 0.06 for coneentrations between 20 and 40%, and 0.09 for eoncentrations between 40 and 60%. This curve indicates that concentrations of phenol as low as 0.3% in phenol-cresol mixtures can be determined with a reasonable degree of accuracy.

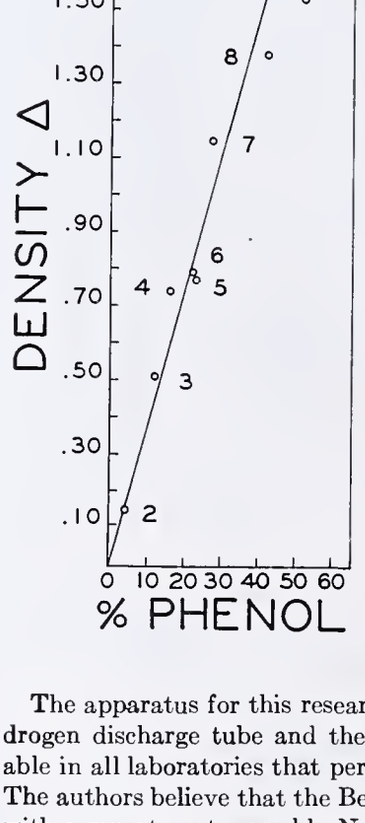


Figure 10. Working Curve for Phenol-Cresol Mixtures Based upon Band *P* of Wave Length 2637 Å.

Density Δ and band *P* are defined in text

The apparatus for this research, with the exception of the hydrogen discharge tube and the quartz absorption cell, is available in all laboratories that perform emission spectrum analysis. The authors believe that the Beckman quartz spectrophotometer with compartment assembly No. 2510 and gas cells 2310-GS-100 can be used for this analysis. This instrument was not available to the authors at the time this work was carried out.

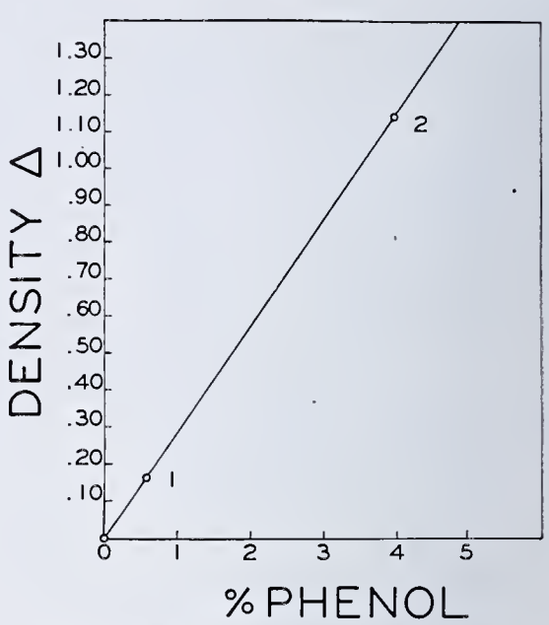


Figure 11. Working Curve for Small Percentages of Phenol in Phenol-Cresol Mixtures

Based upon phenol band A_{PH} of wave length 2750 Å.

Table II. Percentages of Phenol in Synthetic Phenol-Cresol Mixtures and Those Read from Working Curve of Figure 10

Sample	Synthesis %	Analysis %
2	4.0	4
3	12.4	13
4	16.4	19
5	23.1	20
6	21.5	22
7	26.5	31
8	41.5	37

Table III. Analyses of Four Arbitrarily Chosen Samples of Phenol-Cresol Mixtures

Sample		Synthesis %	Analysis %
4	*p*-Cresol	40.1	40
	o-Cresol	21.5	23
6	*p*-Cresol	21.4	20
	o-Cresol	24.3	22
7	*p*-Cresol	43.7	43
	o-Cresol	15.1	17
8	*p*-Cresol	20.7	21
	o-Cresol	21.8	20

ACKNOWLEDGMENT

The cresols used in this investigation were prepared by t Reilly Tar and Chemical Corporation and appeared pure und spectroscopic examination. These were lent by R. J. Willian to whom they were originally given.

The authors wish to thank A. D. Glover for the use of the se sitometer by means of which the characteristic curve was o tained and to acknowledge the continued support of the Resear Institute of the University of Texas.

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Estimation of Moisture in Solventless Double-Base Powders

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Desiccation, cone-and-dish, carbon tetrachloride distillation, and Karl Fischer procedures for the estimation of moisture in ballistite-type powders have been compared. Desiccation over concentrated sulfuric acid has been found satisfactory with respect to accuracy and also the simplest and most reproducible of these procedures, but under certain conditions the other methods are feasible for more rapid determinations.

MOST solventless double-base powder manufactured at the present time is a mixture of nitrocellulose and nitroglycerin to which a few per cent of stabilizers, plasticizers, and inorganic salts have been added. Powders of this type are not notably hygroscopic, but finished samples in equilibrium with their surroundings contain a few tenths of a per cent of moisture. Precise control of the concentration of this constituent is essential to ensure satisfactory behavior of the finished powder. Several procedures for the estimation of moisture in smokeless powders have been reported (*2*, *3*, *4*), many of which are tedious and involved or are not capable of the necessary accuracy. The authors have selected the four procedures that appear most promising and have compared them carefully with regard to accuracy, reproducibility, and ease of operation, using several samples of ballistite-type powder containing approximately 0.2% moisture. The results of these comparisons are presented below, together with specific recommendations based upon them.

EXPERIMENTAL PROCEDURES

DESICCATION PROCEDURE. The desiccation procedure for the estimation of moisture involves storing the powder sample in a desiccator until the weight is constant; the moisture content is calculated from the total loss in weight of the sample.

If the powder sample was in the form of a sheet, it was cut into pieces approximately 1 cm. on a side; if it was in the form of an extruded grain, approximately 1.25 cm. (0.5 inch) were cut from the end of the grain and discarded; successive slices 1 to 2 mm. in thickness were then cut from the exposed surface by means of a guillotine-type cutter. These slices were finally cut into pieces of convenient size.

A 10-gram sample of the freshly cut powder was rapidly weighed into a large dry weighing bottle and placed in a desiccator over concentrated (96%) sulfuric acid. The bottle was tared during the weighing against another bottle of approximately the same size, which was then stored in the desiccator with the sample.

The sample was reweighed every 2 or 3 days until the change between two successive weighings was less than 1 mg., and the percentage of moisture was calculated from the total loss in weight of the system.

CONE-AND-DISH PROCEDURE. The cone-and-dish procedure involves heating the sample to be analyzed in a shallow aluminum dish of special design which is covered with a snugly fitting glass cone (*6*). The successful operation of this method depends upon the assumption that under these conditions the moisture and volatile solvent in the powder diffuse out at the base of the cone, and that any other constituents which are volatilized by the heating condense on the cooler inner surface. Therefore the loss in weight of the entire system is taken to represent the total volatile content of the powder which for a solventless powder may be taken to be the moisture content.

A 10-gram sample of the powder was prepared by the method used for the desiccation procedure described above. This sample was weighed into a cone-and-dish system of standard design, which was tared against another cone-and-dish. The system was placed for 2 hours on a closed bath heated by steam at atmospheric pressure and was then cooled overnight in a desiccator over sulfuric acid and reweighed. The percentage of moisture was calculated from the total loss in weight of the system.

CARBON TETRACHLORIDE DISTILLATION PROCEDURE. The carbon tetrachloride distillation procedure involves refluxing a large sample of powder with carbon tetrachloride and catching the condensed vapors in a trap which retains the water but permits the carbon tetrachloride to return to the boiling flask (*1*).

A 100-gram sample of powder was weighed into a 1-liter round-bottomed flask equipped with a standard-taper joint, and 400 ml. of carbon tetrachloride saturated with water were added. A moisture trap for retaining the water in the condensate was fitted to the flask, and a reflux condenser was mounted on top of the trap. The flask was immersed in a graphite bath which was heated with a 500-watt electrical heater, and the carbon tetrachloride was refluxed until the amount of water in the tube of the moisture trap did not appear to be changing with time. Two to 4 hours were found to be a satisfactory time of refluxing. At the end of the run the heater was shut off and the volume of condensed water was read as the difference between the top of the lower meniscus and the top of the upper meniscus.

A blank determination was made in which 0.20 ml. of water was measured into 400 ml. of carbon tetrachloride saturated with water, and the mixture was refluxed. The percentage of water found in the powder was corrected for the small discrepancy between the amounts of water taken and detected in this blank determination.

KARL FISCHER PROCEDURE. The Karl Fischer reagent is a solution of iodine and sulfur dioxide in pyridine and methanol. This reagent combines with water stoichiometrically (*7*), and the end point can be determined either visually or by the "dead-stop" conductometric method of Foulk and Bawden (*5*).

The grain of powder to be analyzed was cut into slices approximately 1 mm. thick, and the slices were then cut into small pieces. The entire preparation of the sample was carried out as rapidly as possible, and the powder was added to the solvent in the titration flask within 15 to 20 minutes after the first cut had been made on the grain.

A 60-ml. portion of freshly mixed 1 to 1 ether-methanol or 1 to 1 pyridine–ethyl acetate was placed in a titration flask, and 10 ml. of Karl Fischer reagent were added. The mixture was stirred for one minute, and more Karl Fischer reagent was added if it was not still in excess. The solution was finally titrated conductometrically to the anhydrous point with a standard solution of water in methanol, the flask was disconnected from the apparatus, and the 5-gram sample of cut powder was added. The mixture was stirred for a predetermined period of from 30 to 60 minutes, a measured excess of Karl Fischer reagent was added, and after one minute of stirring it was back-titrated with the standard solution of water in methanol. It was found to be important to add enough Karl Fischer reagent so that no less than 3 ml. of water-methanol were required for the back-titration.

A blank determination was made each day. During this determination the flask was opened to the atmosphere for 10 seconds at the time the powder sample would ordinarily be added.

EXPERIMENTAL RESULTS

Several samples of a sheet ballistite (Radford lot JP 198) were analyzed for moisture by the desiccation, cone-and-dish, and carbon tetrachloride procedures; the results of the analyses are presented in Tables I to III and summarized in Table IV. The data illustrate the precision to be expected from the various procedures and the absolute agreement between them.

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Table I. Estimation of Moisture in JP 198 by Desiccation over Sulfuric Acid

Run No.	Percentage Moisture Detected after Desiccation for 48 hours	132 hours	180 hours
1	0.208	0.217	0.218
2	0.205	0.210	0.212
3	0.207	0.214	0.216
4	0.193	0.201	0.201
5	0.194	0.205	0.204
6	0.185	0.201	0.201
			Av. 0.209

Table II. Estimation of Moisture in JP 198 by Cone-and-Dish Procedure

Run No.	Percentage Moisture Detected after 2-hour heating plus 0.5-hour desiccation	2-hour heating plus overnight desiccation
1	0.147	0.178
2	0.158	0.196
3	0.158	0.205
4	0.164	0.221
5	0.168	0.200
6	0.176	0.197
		Av. 0.200

Table III. Estimation of Moisture in JP 198 by Carbon Tetrachloride Distillation

Run No.	Percentage Moisture Detected[a]
1	0.22
2	0.25

[a] A correction of 0.03 ml. was added to volume of water observed bef percentage of moisture was calculated. The value of this correction w obtained from a blank determination with a known amount of water a no powder.

Table V. Estimation of Moisture in JP 198

Procedure	Weight of Sample, Grams	No. of Determinations	Average % Water Detected	Maximum Deviation from Average	Mean Deviation from Average
Desiccation	10	6	0.209	0.009	0.007
Cone-and-dish	10	6	0.200	0.022	0.009
CCl_4 distillation	100	2	0.235	0.015	0.015

Table V. Estimation of Moisture in JP 214

Estimation by Desiccation Run No.	Percentage moisture	Estimation by Karl Fischer Procedure Run No.	Solvent	Percentag moisture
1	0.274	1	Ether-methanol	0.29
2	0.267	2		0.29
3	0.260	3		0.27
4	0.262	4		0.28
5	0.286	5		0.29
6	0.283	6	Pyridine–ethyl acetate	0.29
		7		0.29
Av.	0.272			Av. 0.29

Table VI. Estimation of Moisture in JP 214 after Desiccation an Rehumidification

Description of Sample	Approximate % Relative Humidity of Humidifier	% Gain in Weight on Rehumidification	Percentage Moisture Redesiccation	Cone-and-dish	Karl Fischer
Desiccated	0	...	...	0.079	0.020
Rehumidified	25	0.206	0.218	0.234	0.235
Rehumidified	50	0.324	0.319	0.317	0.342
Rehumidified	75	0.498	0.494	0.480	0.496
Redesiccated	0	...	...	...	0.010

An extruded grain of ballistite (Radford lot JP 214) was analyzed for moisture by the desiccation and Karl Fischer procedures described above, and the results are compared in Table V.

The analyses presented in Tables I to V illustrate the relative amounts of water detected in representative powders by the various procedures, but they do not provide information as to the absolute accuracy of the procedures. In order to secure this information several samples of powder cut from a grain of ballistite (Radford lot JP 214) were desiccated to constant weight, and some of the desiccated samples were analyzed by various procedures. The other desiccated samples were stored in humidifiers over solutions of glycerol and water until they were again constant in weight. These rehumidified samples were then analyzed for moisture. The results of all these experiments are presented in Table VI.

CRITIQUE OF METHODS

Desiccation Procedure. The desiccation procedure is the most reproducible of all of those studied. Although at least 4 days are required for the estimation of moisture in a sample of powder by desiccation, the time per analysis which is required of the analyst is less than that required for any of the other procedures which were studied.

The results of experiments presented in Table VI, in which samples of desiccated powder were analyzed for moisture by titration with Karl Fischer reagent, indicate that the water not removed by desiccation amounts to no more than 0.02% of the powder. Since the Karl Fischer procedure involves complete solution of the powder prior to titration, and since the Karl Fischer and desiccation procedures give comparable results in analyses of powders containing significant amounts of moisture, it appears very unlikely that the water remaining in powder after desiccation is more than the 0.02% indicated by these experiments.

The experiments presented in Table VI indicate that the loss in weight on redesiccation of a rehumidified sample is equal to but not greater than the gain in weight on rehumidification of the sample after the first desiccation. Since the gain in weight on rehumidification can scarcely be due to any material except water, the experiments indicate that no constituents of the powder except water are removed by desiccation. In order to test this conclusion, the acid from a desiccator which had been used in these experiments was tested for nitroglycerin and diphenylamine, but there was no indication of either substance. Therefore the quantities of these constituents lost during desiccation could not have amounted to more than a few hundredths of a per cent of the powder.

All the results indicate that the desiccation procedure for the estimation of moisture in ballistite is simple, accurate, and reproducible; its only disadvantage is the length of time, 4 days, required to complete an analysis.

Cone-and-Dish Procedure. The average of six analyses of JP 198 by the cone-and-dish procedure is in good agreement with the result of six analyses of the same powder by desiccation, but the individual determinations by the cone-and-dish procedure are scattered over a wider range. The data in Table VI suggest that this procedure gives results which are somewhat high for samples containing very little moisture and low for samples containing more than 0.4% of moisture; therefore, it is probable that the success of the procedure for the analysis of ballistites containing 0.2 to 0.4% of moisture is due to a balance between incomplete removal of water and loss through volatilization of other constituents of the powder. The procedure can be recommended only for the rapid but not highly precise analysis of powder samples containing normal amounts of moisture.

Carbon Tetrachloride Distillation Procedure. The carbon tetrachloride distillation procedure yields results rapidly, and errors due to sampling are undoubtedly diminished by the use of a 100-gram sample of powder. However, the results are precise to only about 0.03% of the powder, and the absolute accuracy of the procedure may be somewhat less because of the uncertainties associated with the correction to be applied to the volume of water which is measured. This procedure is suited for control work in which an accuracy of 0.05% is satisfactory.

KARL FISCHER PROCEDURE. The results of the first analyses of powders by means of Karl Fischer reagent scattered over a wide range and indicated considerably more moisture than was detected by the desiccation procedure. The data in Tables V and VI indicate that the moisture detected by the Karl Fischer procedure as finally adopted is no more than 0.02% more than that detected by desiccation, and the results are not sufficiently precise to determine whether there is a significant difference between the percentages of moisture detected by the two procedures. Apparently the Karl Fischer procedure is capable of rapid and accurate estimation of moisture in smokeless powder; however, it requires the use of complicated apparatus and the exercise of special precautions in order to obtain satisfactory results.

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Bromination of Phenols and Phenol Alcohols

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A number of phenols and phenol alcohols were brominated with excess acid bromide–bromate solution at 25°. For each compound the number of positions which brominate is plotted as a function of the bromine excess. The effect of acid concentration, bromination time, and temperature on the extent of bromination is described. While some alkylated phenols brominate quantitatively regardless of the bromine excess used, other alkylated phenols overbrominate to an extent which varies with the magnitude of the bromine excess. The hydroxymethyl groups of phenol alcohols differ widely in the ease with which they are displaced by bromine.

ALTHOUGH the quantitative bromination of phenols by acid bromide-bromate solution, first described by Koppeschaar (*11*) in 1876, has been the subject of numerous investigations, there is much about this reaction that is still not clear. The method is based upon the general assumption that aqueous nascent bromine will replace quantitatively only those hydrogen atoms ortho or para to the hydroxyl group of phenols. It has been found, however, that the presence of certain groups other than hydrogen in the ortho or para positions gives rise to anomalous results.

Numerous experiments by Allen (*1*) and others proved that salicylic acid brominates with the splitting off of the carboxy group and the formation of carbon dioxide and tribromophenol. The bromination of sulfanilic acid was studied by Callan and Henderson (*3*) who, in agreement with previous investigators, found that the sulfo group is eliminated and tribromoaniline is formed. Francis and Hill (*9*) established that the formyl group in *o*- and *p*-hydroxybenzaldehyde is displaced during bromination with the evolution of carbon monoxide and the formation of tribromophenol. Francis (*7*) found that when saligenin is brominated the hydroxymethyl group is displaced and formaldehyde is produced. The displacement of the COOH, HSO_3, CHO, and CH_2OH groups in the phenols studied by the above investigators could be made quantitative, or could be completely inhibited, depending upon the bromination conditions.

There is considerable disagreement in the literature on the accuracy with which alkylated phenols can be determined by bromination. Thus, Fox and Barker (*6*) using the bromide-bromate method observed that phenol and *m*-cresol absorb exactly 3 moles of bromine, but that *o*- and *p*-cresol absorb somewhat more than 2 moles of bromine. Day and Taggart (*4*) likewise found Koppeschaar's method unsatisfactory for the determination of *o*- and *p*-cresol, although phenol, *m*-cresol, β-naphthol, thymol, resorcinol, several nitrophenols, *p*-chlorophenol, salicylic acid, *m*-hydroxybenzoic acid, and a number of salicylates gave good results. In a recent study of the bromide-bromate method, Sprung (*17*) found that phenols which contain substituents in the meta position, or phenols which contain secondary or tertiary alkyl groups in the ortho or para positions, brominate quantitatively. However, phenols which contain primary alkyl groups in the ortho or para positions give results which may be 10 to 150% high. Francis and Hill (*8*) contend that the overbromination of cresols and xylenols observed by Sprung does not occur if only a slight excess of bromine is used, and if the bromination time is very short.

It was the purpose of the investigation reported here to study further Koppeschaar's bromination method with the aim of applying it to the measurement of the rates of condensation of phenols with formaldehyde. For this application it is necessary to know accurately how phenols containing alkyl, hydroxymethyl, and methylene groups brominate. Since no information about the quantitative bromination of phenol alcohols, other than saligenin, could be found in the literature, a number of phenol alcohols were prepared and brominated. The related phenols, as well as *o*-cresol, *m*-cresol, and three isomeric dihydroxydiphenylmethanes were likewise brominated to ascertain the accuracy with which they could be determined. The effect of the magnitude of the bromine excess on the extent of bromination of each phenol and phenol alcohol was studied in detail. The effects of acid concentration, bromination time, and temperature were also investigated in the cases of *p*-cresol and 2-hydroxy-α^1,α^3-mesitylenediol.

SOLUTIONS AND REAGENTS

Sodium Thiosulfate, 0.1 *N*.

Bromide-Bromate Solution, 0.1 *N*. Potassium bromate (5.6 grams) and potassium bromide (30 grams) dissolved in distilled water and made up to 2 liters.

Hydrochloric Acid. Concentrated acid, specific gravity 1.19.

Potassium Iodide, 10% aqueous solution.

Starch Solution, 1% aqueous solution.

Phenol. C.P. grade phenol redistilled three times; melting point 40° C.

Saligenin. Prepared by the reduction of *o*-hydroxybenzaldehyde with sodium amalgam according to the procedure of Lapworth and Schoesmith (*12*). Recrystallized four times from

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benzene and three times from carbon tetrachloride; melting point 84° C.

p-Hydroxybenzyl Alcohol. Obtained from phenol-formaldehyde resins by sublimation (*16*). Purified by fractional crystallization from benzene and several recrystallizations from water; melting point 121°.

o-Cresol. Pure *o*-cresol from The Barrett Division redistilled three times; melting point 31°.

m-Cresol. Practical grade material from the Eastman Kodak Company purified by the method of Fox and Barker (*5*); melting point 10°.

p-Cresol. *p*-Cresol (99 to 100%) from The Barrett Division purified by the method of Fox and Barker (*5*); melting point 33°.

2-Hydroxy-α^1,α^3-mesitylenediol. Prepared from *p*-cresol and formaldehyde according to Auwers (*2*) and Ullmann and Brittner (*19*). Recrystallized once from ethyl acetate and once from benzene; melting point 128°.

p-Ethylphenol. A sample from the Reilly Tar & Chemical Corp. redistilled once; melting point 43°.

5-Ethyl-2-hydroxy-α^1,α^3-xylenediol. Prepared from *p*-ethylphenol and formaldehyde (*18*). Recrystallized once from benzene; melting point 84°.

p-Isopropylphenol. A sample from Sharples Chemicals, Inc., recrystallized twice from petroleum ether; melting point 60°.

2-Hydroxy-5-isopropyl-α^1,α^3-xylenediol. Prepared from *p*-isopropylphenol and formaldehyde (*18*). Recrystallized once from benzene; melting point 125°.

p-Cyclohexylphenol. A commercial sample from The Dow Chemical Company recrystallized once from heptane; melting point 130°.

5-Cyclohexyl-2-hydroxy-α^1,α^3-xylenediol. Prepared from *p*-cyclohexylphenol and formaldehyde (*20*). Recrystallized once from benzene; melting point 107°.

p-(1,1,3,3-Tetramethylbutyl)-phenol. A commercial sample from The Resinous Products & Chemical Company recrystallized once from petroleum ether; melting point 84°.

2-Hydroxy-5-(1,1,3,3-tetramethylbutyl)-α^1,α^3-xylenediol. Prepared from *p*-(1,1,3,3-tetramethylbutyl)-phenol and formaldehyde (*14*). Recrystallized three times from petroleum ether; melting point 71°.

4-Chloro-*m*-cresol. A commercial sample from The Barrett Division recrystallized once from petroleum ether; melting point 64°.

6-Chloro-3-hydroxy-α^2,α^4-pseudocumenediol. Prepared from 4-chloro-*m*-cresol and formaldehyde (*13*). Recrystallized twice from 1,2-dichloroethane; melting point 131°.

2,2′-Dihydroxydiphenylmethane. A pure sample furnished by Givaudan-Delawanna, Inc.; melting point 118°.

2,4′-Dihydroxydiphenylmethane. Isolated from an acid-catalyzed

Table I. Bromination of Some Phenols and Phenol Alcohols

Acidity, 0.75 *N*
Temperature, 25° C.
Time exposed to bromine, 5 minutes

Phenol	Concentration of Phenol Solution	Br Added	$Na_2S_2O_3$ Required	Net Br Absorbed	Residual Br Excess	No. of Reactive Positions Found
	G./l.	*Milliequivalents*	*Milliequivalents*	*Milliequivalents*	*% Br absorbed*	
Phenol	1.507	2.496	0.088	2.407	3.66	3.01
	1.507	2.504	0.098	2.406	4.07	3.01
	0.9490	1.702	0.188	1.514	12.4	3.00
	0.9490	1.922	0.406	1.516	26.8	3.01
	0.9490	2.338	0.817	1.521	53.7	3.02
Saligenin	1.047	1.244	0.013	1.231	1.06	2.92
	1.047	1.282	0.043	1.239	3.47	2.94
	1.047	1.322	0.082	1.240	6.61	2.94
	1.047	1.540	0.282	1.258	22.4	2.98
	1.047	1.736	0.473	1.263	37.5	2.99
	1.047	1.933	0.653	1.280	51.0	3.03
	1.047	2.031	0.754	1.277	59.0	3.03
p-Hydroxybenzyl alcohol	1.004	1.195	0.038	1.157	3.28	2.86
	1.004	1.262	0.099	1.163	8.51	2.88
	1.004	1.793	0.587	1.206	48.7	2.98
	1.004	2.340	1.119	1.221	91.6	3.02
o-Cresol	1.669	1.766	0.156	1.610	9.69	2.09
	1.669	1.803	0.184	1.619	11.4	2.10
	0.8251	0.965	0.133	0.832	16.0	2.18
	0.8251	1.284	0.308	0.976	31.6	2.56
	0.8251	1.440	0.408	1.032	39.5	2.70
	0.8251	1.682	0.601	1.081	55.6	2.83
	0.8251	2.008	0.883	1.125	78.5	2.95
	0.8251	2.380	1.252	1.128	111	2.96
m-Cresol	0.5902	0.837	0.029	0.808	3.59	2.96
	1.146	1.664	0.100	1.564	6.39	2.95
	1.002	1.461	0.090	1.371	6.56	2.96
	0.5902	0.977	0.172	0.805	21.4	2.95
	1.002	1.717	0.344	1.373	25.1	2.96
	1.146	2.067	0.479	1.588	30.2	3.00
	0.5902	1.124	0.302	0.822	36.7	3.01
	1.146	2.287	0.693	1.594	43.5	3.01
	1.002	2.068	0.670	1.398	47.9	3.02
	1.146	2.581	0.974	1.607	60.6	3.03
	0.5902	1.315	0.506	0.809	62.5	2.96
	0.5902	1.407	0.592	0.815	72.6	2.99
	0.5902	1.487	0.666	0.821	81.1	3.01
p-Cresol	1.050	1.033	0.068	0.965	7.05	1.99
	0.9364	0.941	0.076	0.865	8.79	2.00
	1.050	1.361	0.281	1.080	26.0	2.22
	1.050	1.566	0.460	1.106	41.6	2.28
	0.9364	1.569	0.585	0.984	59.5	2.27
	1.050	2.000	0.899	1.101	81.7	2.27
p-Ethylphenol	1.052	0.796	0.063	0.733	8.59	1.70
	1.052	0.931	0.129	0.802	16.1	1.86
	1.052	1.119	0.248	0.871	28.5	2.02
	1.052	1.527	0.586	0.941	62.3	2.19
	1.052	2.039	1.066	0.973	110	2.26
p-Isopropylphenol	1.337	0.915	0.069	0.846	8.16	1.72
	1.337	1.152	0.205	0.947	21.6	1.93
	1.337	1.322	0.307	1.015	30.2	2.07
	1.337	1.524	0.470	1.054	44.6	2.15
	1.337	1.910	0.734	1.176	62.4	2.40
p-Cyclohexylphenol	2.142	1.248	0.038	1.210	3.14	1.99
	2.142	1.369	0.153	1.216	12.6	2.00
	2.142	1.453	0.242	1.211	20.0	1.99
	2.142	1.560	0.322	1.238	26.0	2.04
	2.142	1.867	0.680	1.187	57.3	1.95
p-(1,1,3,3-Tetramethylbutyl)-phenol	3.764	1.890	0.064	1.826	3.50	2.00
	4.025	2.139	0.195	1.944	10.0	1.99
	4.025	2.209	0.267	1.942	13.7	1.99
	4.025	2.204	0.265	1.939	13.7	1.99
	3.764	2.267	0.456	1.811	25.2	1.99
	3.764	2.498	0.673	1.825	36.9	2.00
4-Chloro-*m*-cresol	2.101	1.526	0.051	1.475	3.46	2.00
	1.438	1.042	0.046	0.996	4.62	1.98
	2.101	1.666	0.188	1.478	12.7	2.01
	2.101	1.763	0.273	1.490	18.3	2.02
	1.438	1.237	0.242	0.995	24.3	1.97
	2.101	1.864	0.369	1.495	24.7	2.03
	2.101	1.920	0.427	1.493	28.6	2.03
	2.101	2.009	0.524	1.485	35.3	2.02
	1.438	1.379	0.382	0.997	38.3	1.98
	1.438	1.501	0.487	1.014	48.0	2.01
	1.438	1.641	0.639	1.002	63.8	1.99
	1.438	1.761	0.751	1.010	74.4	2.00
2-Hydroxy-α^1,α^3-mesitylenediol	3.027	1.210	0.053	1.157	4.58	1.29
	2.718	1.064	0.051	1.013	5.03	1.25
	2.687	1.180	0.124	1.056	11.7	1.32
	3.027	1.480	0.254	1.226	20.7	1.36
	2.718	1.373	0.291	1.082	26.9	1.34
	2.687	1.407	0.335	1.072	31.3	1.34
	2.687	1.523	0.445	1.078	41.3	1.35
	2.718	1.636	0.522	1.114	46.9	1.38
	3.024	1.976	0.694	1.282	54.1	1.43
	2.718	1.941	0.781	1.160	67.3	1.44
5-Ethyl-2-hydroxy-α^1,α^3-xylenediol	1.683	0.778	0.034	0.744	4.57	1.61
	1.683	0.908	0.117	0.791	14.8	1.71
	1.683	1.055	0.237	0.818	29.0	1.77
	1.683	1.218	0.385	0.833	46.2	1.80
	1.683	1.683	0.836	0.847	98.7	1.83

Table I (Continued).

Phenol	Concentration of Phenol Solution	Br Added	$Na_2S_2O_3$ Required	Net Br Absorbed	Residual Br Excess	No. of Reactive Positions Found
	G./l.	*Milliequivalents*	*Milliequivalents*	*Milliequivalents*	*% Br absorbed*	
5-Hydroxy-5-isopropyl-α^1,α^3-xylenediol	2.020	0.836	0.051	0.785	6.50	1.53
	2.020	0.990	0.132	0.858	15.4	1.67
	2.020	1.199	0.338	0.861	39.3	1.67
	2.020	1.382	0.504	0.878	57.4	1.71
	2.020	1.549	0.655	0.894	73.3	1.74
5-Cyclohexyl-2-hydroxy-α^1,α^3-xylenediol	2.610	0.797	0.093	0.704	13.2	1.28
	2.610	0.930	0.146	0.784	18.6	1.42
	2.610	1.010	0.205	0.805	25.5	1.46
	2.610	1.082	0.283	0.799	35.4	1.45
	2.610	1.295	0.448	0.847	52.9	1.53
	2.610	1.460	0.626	0.834	75.1	1.51
5-Hydroxy-5-(1,1,3,3-tetramethylbutyl)-α^1,α^3-xylenediol	3.708	1.295	0.082	1.213	6.76	1.74
	3.284	1.239	0.113	1.126	10.0	1.83
	3.708	1.416	0.162	1.254	12.9	1.80
	3.284	1.358	0.202	1.156	17.5	1.88
	3.708	1.524	0.249	1.275	19.5	1.83
	3.284	1.534	0.346	1.188	29.1	1.93
	3.284	1.663	0.477	1.186	40.2	1.92
	3.708	1.938	0.573	1.365	42.0	1.96
	3.284	1.881	0.671	1.210	55.5	1.96
	3.284	2.114	0.899	1.215	74.0	1.97
6-Chloro-3-hydroxy-α^2,α^4-pseudocumenediol	3.017	1.471	0.051	1.420	3.59	1.91
	3.017	1.450	0.053	1.397	3.79	1.88
	3.017	1.610	0.162	1.448	11.2	1.95
	3.017	1.717	0.267	1.450	18.4	1.95
	3.017	1.894	0.434	1.460	29.7	1.96
	3.017	2.081	0.598	1.483	40.3	1.99
	3.017	2.293	0.817	1.476	55.4	1.98
	3.017	2.532	1.044	1.488	70.2	2.00
2,2′-Dihydroxydiphenylmethane	1.362	1.048	0.029	1.019	2.85	3.00
	1.362	1.224	0.177	1.047	16.9	3.08
	1.362	1.341	0.287	1.054	27.2	3.10
	1.362	1.742	0.652	1.090	59.8	3.20
4,4′-Dihydroxydiphenylmethane	1.403	1.323	0.027	1.296	2.08	3.70
	1.403	1.514	0.162	1.352	12.0	3.86
	1.403	1.784	0.424	1.360	31.2	3.88
	1.403	2.037	0.666	1.371	48.6	3.91
	1.403	2.269	0.878	1.391	63.1	3.97
4,4′-Dihydroxydiphenylmethane	1.297	1.349	0.065	1.284	5.06	3.96
	1.297	1.524	0.226	1.298	17.4	4.01
	1.297	1.786	0.466	1.320	35.3	4.08
	1.297	2.027	0.688	1.339	51.4	4.13
	1.297	2.525	1.154	1.371	84.2	4.23

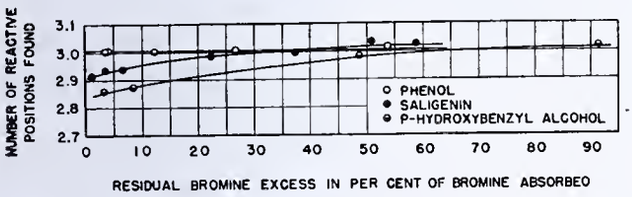

Figure 1. Bromination of Phenol, Saligenin, and *p*-Hydroxybenzyl Alcohol

Acidity, 0.75 *N*
Temperature, 25° C.
Time exposed to bromine, 5 minutes

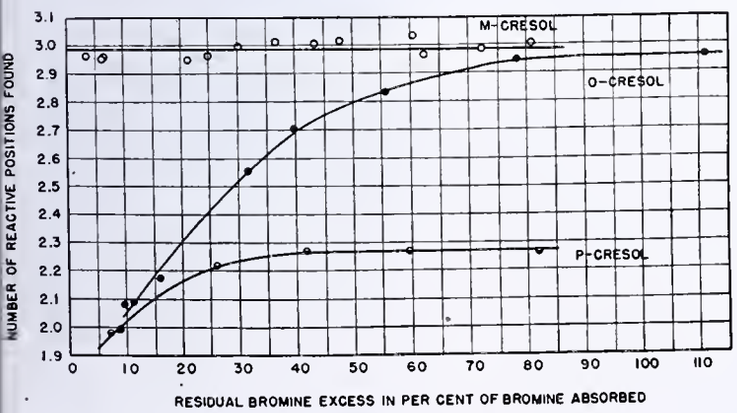

Figure 2. Bromination of *o*-, *m*-, and *p*-Cresol

Acidity, 0.75 *N*
Temperature, 25° C.
Time exposed to bromine, 5 minutes

phenol-formaldehyde resin by vacuum distillation according to Koebner (*10*). Recrystallized twice from water; melting point 118°.

4,4′-Dihydroxydiphenylmethane. A pure sample supplied by Givaudan-Delawanna, Inc.; melting point 162°.

PROCEDURE

Koppeschaar's method (*11*) as modified by Redman, Weith, and Brock (*15*) was essentially the method used. An accurately weighed quantity of the phenol was dissolved in distilled water containing sufficient sodium hydroxide (about 1 gram) to effect solution, and the solution was made up to 1 liter. A 25-ml. aliquot of the phenol solution was transferred to a 250-ml. glass-stoppered iodine flask, to which were added 25 ml. (or more as indicated below) of water and 5 ml. of concentrated hydrochloric acid. Enough bromide-bromate solution was then run in from a buret to give the desired bromine excess. The flask was quickly stoppered and shaken intermittently for 5 minutes. Ten milliliters of potassium iodide solution were then added to the flask, care being taken to avoid loss of bromine while the stopper was being lifted. The stopper was replaced and the solution again shaken intermittently for 5 minutes. The stopper and sides of the flask were washed down with water, and the solution was titrated with sodium thiosulfate, using starch solution as the indicator. A blank was run on 25 ml. of distilled water. All the brominations were carried out at a temperature of 25° except where otherwise indicated. The acidity of the mixture in the iodine flask after the addition of the bromide-bromate solution was maintained between 0.7 and 0.8 *N* by keeping the sum of the bromide-bromate solution and diluent water added at about 50 ml. Thus, when it was planned to add only 15 ml. of bromide-bromate solution to obtain the desired bromine excess, 35 instead of 25 ml. of water were added.

Aqueous solutions of *p*-(1,1,3,3-tetramethylbutyl)-phenol and 2-hydroxy-5-(1,1,3,3-tetramethylbutyl)-α^1,α^3-xylenediol could not be made, because of the insolubility of their sodium salts. Consequently a weighed amount of each of these phenols was dissolved in 600 ml. of acetone-free reagent grade methanol, and the solution made up to 1 liter with distilled water. In order to avoid precipitation, the aliquots of these solutions were diluted with 25 ml. of methanol instead of water prior to bromination. A series of blanks was run on an aqueous methanol solution with various bromine excesses, and a correction curve for the presence of the methanol was constructed. Acetone-free methanol was found to brominate to an almost negligible extent under the conditions used in this work.

Although aqueous solutions of *p*-cyclohexylphenol and 5-cyclohexyl-2-hydroxy-α^1,α^3-xylenediol could be and were prepared, the addition of hydrochloric acid to the aliquots of these phenols caused them to precipitate, with the result that subsequent bromination was found to be incomplete. This difficulty was overcome by diluting the aliquots with methanol instead of water, and correcting for the presence of the methanol by means of the correction curve discussed above.

With a few phenols, such as *p*-ethylphenol and 4-chloro-*m*-cresol, the use of a large excess of bromine caused the end point to reappear shortly after the analysis had been completed. In such cases thiosulfate solution was added until the end point became stable.

Brominations at 10° and 40° were carried out in a constant-

Table II. Effect of Acidity on Extent of Bromination

Temperature, 25° C.
Time exposed to bromine, 5 minutes

Phenol	Concentration of Phenol Solution *G./l.*	Acid Normality	Br Added *Milliequivalents*	$Na_2S_2O_3$ Required *Milliequivalent*	Net Br Absorbed *Milliequivalents*	Residual Br Excess *% Br absorbed*	No. of Reactive Positions Found
p-Cresol	0.9364	0.35	0.903	0.113	0.790	14.3	1.82
	0.9364	0.35	1.042	0.215	0.827	26.0	1.91
	0.9364	0.35	1.272	0.388	0.884	43.9	2.04
	0.9364	0.35	1.491	0.595	0.896	66.4	2.07
	0.9364	0.35	1.708	0.817	0.891	91.7	2.06
	1.050	2.0	0.947	0.018	0.929	1.94	1.91
	0.9364	2.0	0.991	0.128	0.863	14.8	1.99
	1.050	2.0	1.250	0.230	1.020	22.5	2.10
	1.050	2.0	1.465	0.436	1.029	42.4	2.12
	1.050	2.0	1.579	0.518	1.061	48.8	2.19
	0.9364	2.0	1.878	0.909	0.969	93.8	2.24
2-Hydroxy-α^1,α^3-mesitylenediol	2.687	0.35	0.952	0.047	0.905	5.19	1.13
	2.687	0.35	1.062	0.146	0.916	15.9	1.15
	2.687	0.35	1.224	0.298	0.926	32.2	1.16
	2.687	0.35	1.376	0.425	0.951	44.7	1.19
	2.687	0.35	1.590	0.652	0.938	69.5	1.17
	2.687	2.0	1.225	0.043	1.182	3.64	1.48
	2.687	2.0	1.352	0.064	1.288	4.97	1.61
	2.687	2.0	1.554	0.173	1.381	12.5	1.73
	2.687	2.0	2.001	0.558	1.443	38.7	1.81
	2.718	2.0	2.472	0.918	1.554	59.1	1.92

Table III. Effect of Temperature on Extent of Bromination

Acidity, 0.75 *N*
Time exposed to bromine, 5 minutes

Phenol	Concentration of Phenol Solution *G./l.*	Temperature *° C.*	Br Added *Milliequivalents*	$Na_2S_2O_3$ Required *Milliequivalent*	Net Br Absorbed *Milliequivalents*	Residual Br Excess *% Br absorbed*	No. of Reactive Positions Found
p-Cresol	0.9364	10	0.842	0.069	0.773	8.93	1.79
	0.9364	10	0.957	0.160	0.797	20.1	1.84
	0.9364	10	1.106	0.277	0.829	33.4	1.91
	0.9364	10	1.325	0.451	0.874	51.6	2.02
	0.9364	10	1.512	0.643	0.869	74.0	2.01
	0.9364	40	1.046	0.066	0.980	6.73	2.26
	0.9364	40	1.162	0.126	1.036	12.2	2.39
	0.9364	40	1.472	0.336	1.136	29.6	2.62
	0.9364	40	1.696	0.531	1.165	45.6	2.69
	0.9364	40	1.922	0.734	1.188	61.8	2.74
2-Hydroxy-α^1,α^3-mesitylenediol	2.687	10	0.959	0.087	0.872	9.98	1.09
	2.687	10	1.067	0.119	0.948	12.6	1.19
	2.687	10	1.218	0.219	0.999	21.9	1.25
	2.687	10	1.379	0.374	1.005	37.2	1.26
	2.687	10	1.588	0.536	1.052	51.0	1.32
	2.687	10	1.803	0.707	1.096	64.5	1.37
	2.687	40	1.012	0.026	0.986	2.64	1.23
	2.687	40	1.162	0.080	1.082	7.39	1.36
	2.687	40	1.398	0.282	1.116	25.3	1.40
	2.687	40	1.595	0.449	1.146	39.2	1.44
	2.687	40	1.845	0.645	1.200	53.8	1.50

Table IV. Effect of Bromination Time on Extent of Bromination

Acidity, 0.75 *N*
Temperature, 25° C.

Phenol	Concentration of Phenol Solution *G./l.*	Bromination Time *Min.*	Br Added *Milliequivalents*	$Na_2S_2O_3$ Required *Milliequivalent*	Net Br Absorbed *Milliequivalents*	Residual Br Excess *% Br absorbed*	No. of Reactive Positions Found
p-Cresol	1.050	1	0.864	0.064	0.800	8.00	1.65
	1.050	1	0.948	0.098	0.850	11.5	1.75
	1.050	1	1.159	0.247	0.912	27.1	1.88
	1.050	1	1.479	0.525	0.954	55.0	1.96
	1.050	1	1.903	0.940	0.963	97.6	1.98
	1.050	10	1.096	0.125	0.971	12.9	2.00
	1.050	10	1.157	0.157	1.000	15.7	2.06
	1.050	10	1.247	0.204	1.043	19.6	2.15
	1.050	10	1.373	0.296	1.077	27.5	2.22
	1.050	10	1.903	0.811	1.092	74.3	2.25
2-Hydroxy-α^1,α^3-mesitylenediol	2.687	1	0.925	0.057	0.868	6.57	1.09
	2.687	1	1.032	0.109	0.923	11.8	1.16
	2.687	1	1.324	0.325	0.999	32.5	1.25
	2.687	1	1.563	0.526	1.037	50.7	1.30
	2.687	1	1.782	0.754	1.028	73.3	1.29
	2.687	10	1.016	0.033	0.983	3.36	1.23
	2.687	10	1.185	0.084	1.101	7.63	1.38
	2.687	10	1.378	0.268	1.110	24.1	1.39
	2.687	10	1.570	0.467	1.103	42.3	1.38
	2.687	10	1.816	0.673	1.143	58.9	1.43

temperature bath up to the poir of addition of the potassiur iodide.

The number of reactive pos tions, R, was calculated from th formula

$$R = \frac{E\,M}{50\,C}$$

where E is the milliequivalents o bromine absorbed, M is the mo lecular weight of the phenol, an C is the concentration of the pheno solution in grams per liter.

The residual bromine excess i per cent was obtained by divid ing the milliequivalents of sodiun thiosulfate used (which is equa to the milliequivalents of unre acted bromine) by the milliequiva lents of bromine absorbed, and multiplying the result by 100.

RESULTS AND DISCUSSION

Phenol, Saligenin, and *p*-Hydroxybenzyl Alcohol. Figure 1 shows that phenol brominates quantitatively at three positions, regardless of the size of the bromine excess. When a considerable bromine excess is used, three reactive positions are also found for saligenin and *p*-hydroxybenzyl alcohol, thereby indicating quantitative displacement of the hydroxymethyl groups by bromine; but the presence of a greater bromine excess than that required for complete displacement produces no further bromination.

Cresols. Although *o*- and *p*-cresol each absorb about 2 moles of bromine when the bromine excess is small, additional absorption of bromine occurs with larger bromine excesses (Figure 2). The curves show clearly that *o*-cresol overbrominates to a much greater extent than does *p*-cresol. In view of these results, the discrepancies in the literature regarding the accuracy with which *o*- and *p*-cresol can be determined are understandable. For, depending upon the bromine excess that is used, a whole range of results will be obtained. *m*-Cresol, however, brominates quantitatively at three positions over a wide range of bromine excesses.

The reason for the overbromination of the cresols, and of other phenols discussed below, is not clear. Sprung favors the theory that substitution occurs in the

alkyl groups ortho or para to the phenolic hydroxyl group. Another possible explanation is that methylene quinones are formed, thereby consuming additional bromine over that theoretically required for substitution in the free ortho or para positions. No direct evidence has yet been presented in support of either of these theories.

Alkylated Phenols and Their Hydroxymethyl Derivatives. An examination of Figure 3 reveals that only *p*-(1,1,3,3-tetramethylbutyl)-phenol, *p*-cyclohexylphenol, and 4-chloro-*m*-cresol show two reactive positions regardless of the size of the bromine excess. *p*-Cresol, *p*-ethylphenol, and *p*-isopropylphenol brominate abnormally; the larger the bromine excess, the greater is the number of reactive positions that are found.

Before attempting to explain or generalize these results, it seems desirable to point out that the importance of these data lies not so much in the absolute values as in the trends that are revealed. In other words, while it may be useful to know that 2.96 reactive positions are found for *o*-cresol when the residual bromine excess is 100%, it is more important to know that in the presence of excess bromine *o*-cresol readily absorbs more than 2 moles of bromine, the overbromination depending upon the magnitude of the bromine excess. Aside from the theoretical value, knowing whether a phenol tends to overbrominate is a prerequisite for the establishment of an accurate quantitative bromination method for the phenol. The results obtained for the phenols studied lead to the following generalizations, which extend and modify those proposed by Sprung. Phenols having primary or secondary alkyl groups ortho or para to the phenolic hydroxyl group tend to overbrominate in the presence of excess bromine, the extent of overbromination depending upon the magnitude of the bromine excess. Phenols having ortho, meta, or para tertiary alkyl groups brominate quantitatively, regardless of how large the bromine excess is.

p-Cyclohexylphenol is an exceptional case, for although it has a secondary alkyl group, it brominates quantitatively. The cyclohexyl group possibly introduces sufficient steric hindrance to prevent overbromination.

Figure 3 also shows that only with 2-hydroxy-5-(1,1,3,3-tetramethylbutyl)-α^1,α^3-xylenediol and 6-chloro-3-hydroxy-α^2,α^4-pseudocumenediol does quantitative displacement of both hydroxymethyl groups by bromine take place, and even with these dialcohols large bromine excesses are required for complete displacement. Only partial elimination of the hydroxymethyl groups occurs when 2-hydroxy-α^1,α^3-mesitylenediol, 5-ethyl-2-hydroxy-α^1,α^3-xylenediol, 2-hydroxy-5-isopropyl-α^1,α^3-xylenediol, and 5-cyclohexyl-2-hydroxy-α^1,α^3-xylenediol are brominated. The data concerning these dialcohols are not only of value in indicating the relative ease with which the hydroxymethyl groups are displaced, but they also demonstrate clearly that it cannot be assumed that bromination conditions under

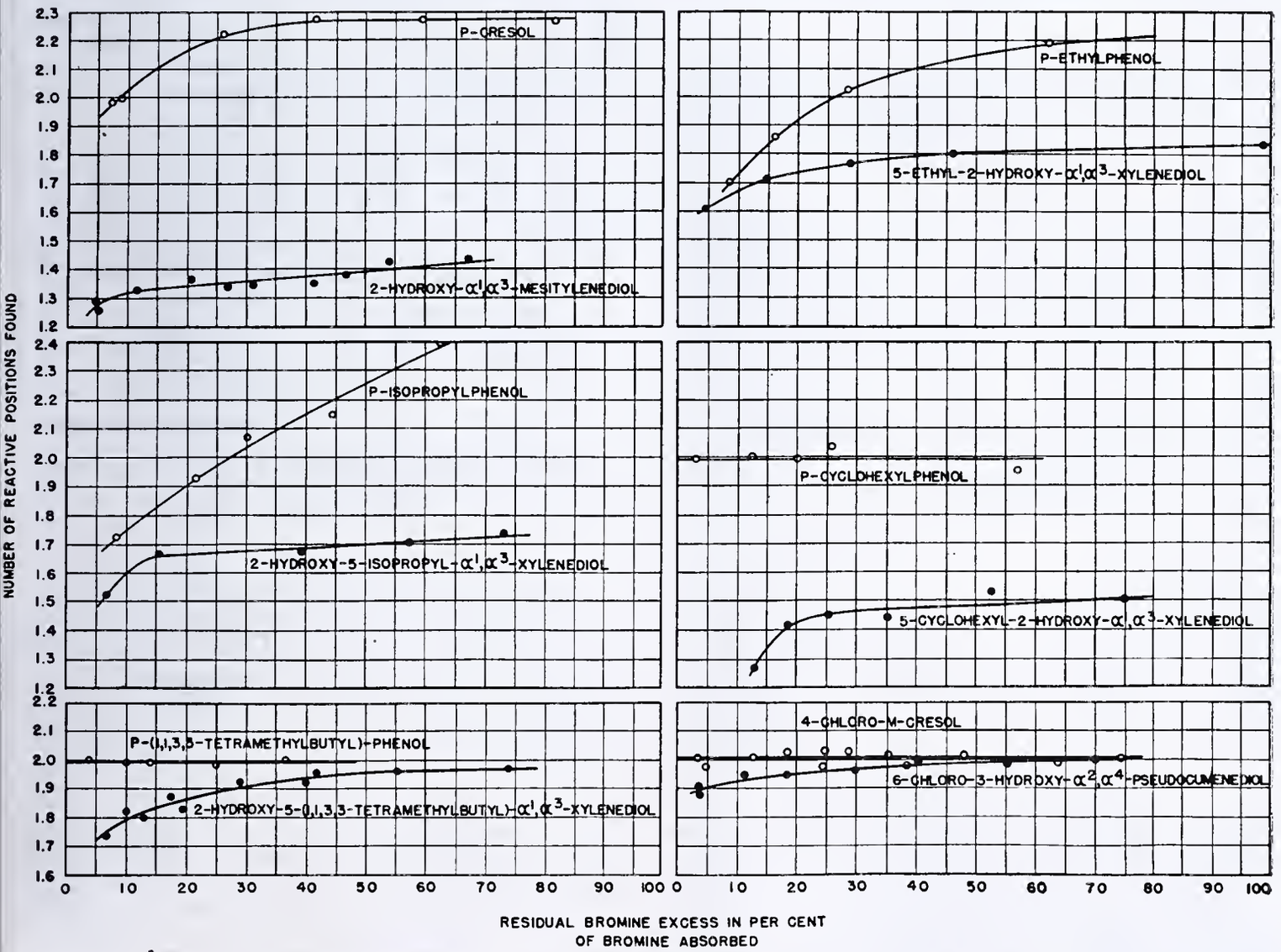

Figure 3. Bromination of Some Alkylated Phenols and Their Hydroxymethyl Derivatives

Acidity, 0.75 *N*
Temperature, 25° C.
Time exposed to bromine, 5 minutes

which an alkylated phenol brominates quantitatively will give quantitative bromination of the dialcohol of this phenol. It appears that para-alkylated phenol dialcohols whose alkyl groups are tertiary lose their 2 hydroxymethyl groups during bromination much more easily than do para-alkylated phenol dialcohols whose alkyl groups are primary or secondary.

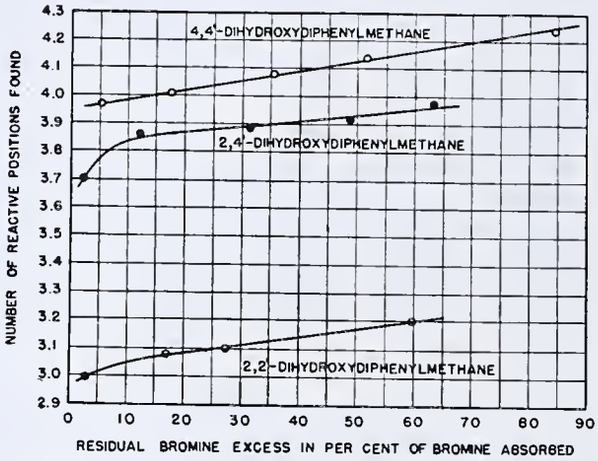

Figure 4. Bromination of 2,2′-, 2,4′-, and 4,4′-Dihydroxydiphenylmethane

Acidity, 0.75 *N*
Temperature, 25° C.
Time exposed to bromine, 5 minutes

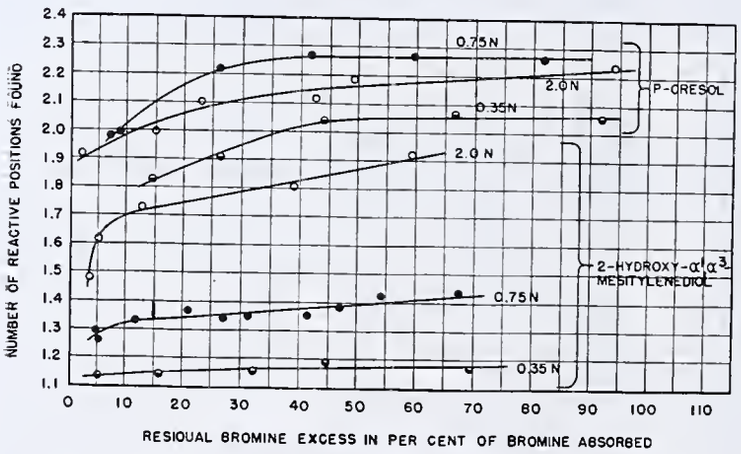

Figure 5. Effect of Acidity on Extent of Bromination

Temperature, 25° C.
Time exposed to bromine, 5 minutes

Dihydroxydiphenylmethanes. The 2,2′-, 2,4′-, and 4,4′-isomers differ widely in the extent to which they brominate, as shown in Figure 4. While 4,4′-dihydroxydiphenylmethane absorbs 4 moles of bromine with small bromine excesses, and overbrominates with greater bromine excesses, a large excess of bromine is needed for the 2,4′-isomer to brominate at 4 positions. The 2,2′-isomer underbrominates even when a large bromine excess is present. These results indicate that dihydroxydiphenylmethanes brominate more readily at positions ortho to the phenolic hydroxyl group than at para positions. Judging from these results, bromination can hardly be regarded as an accurate method for determining the molecular weight of dihydroxydiphenylmethanes.

Acidity Effect. As shown in Figure 5, the acid normality of the acidified phenol solution after the addition of the bromide-bromate solution has an appreciable effect on the extent of bromination of *p*-cresol. Reducing the acidity from 0.75 *N* to 0.35 *N* decreases the amount of overbromination of *p*-cresol, but the differences which result from increasing the acidity from 0.75 *N* to 2.0 *N* may be due more likely to experimental error than to a significant trend.

The acidity has an even more pronounced effect on the displacement of the hydroxymethyl groups of 2-hydroxy-α^1,α^3-mesitylenediol by bromine. The higher the acid normality is, the greater is the displacement of hydroxymethyl groups.

Temperature Effect. Figure 6 shows that the extent to which *p*-cresol brominates depends greatly upon the temperature. At 10° no overbromination takes place even in the presence of a large excess of bromine. The displacement of the hydroxymethyl groups of 2-hydroxy-α^1,α^3-mesitylenediol is similarly retarded by low temperatures, although the magnitude of the temperature effect is not so great as it is in the case of *p*-cresol.

Time Effect. A change in the bromination time from 1 minute to 5 minutes produces a significant increase in the number of reactive positions found for *p*-cresol and for 2-hydroxy-α^1,α^3-mesitylenediol, as may be seen from Figure 7. Increasing the bromination time beyond 5 minutes does not affect the results appreciably.

No universal bromination procedure can be recommended at this time which can be used with confidence for the quantitative bromination of phenols, phenol alcohols, dihydroxydiphenyl-

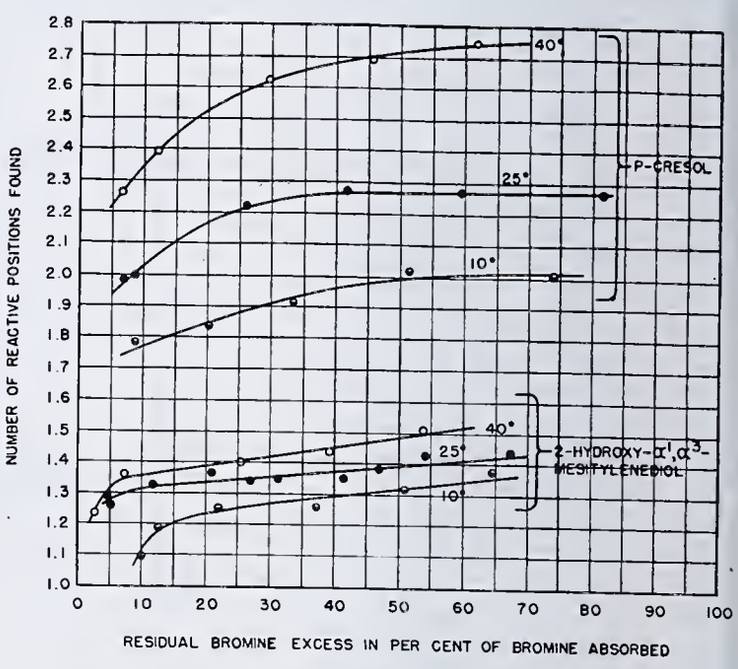

Figure 6. Effect of Temperature on Extent of Bromination

Acidity, 0.75 *N*
Time exposed to bromine, 5 minutes

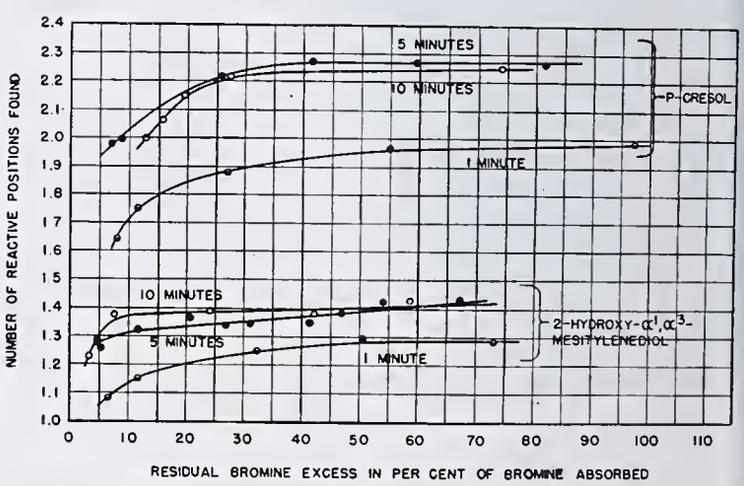

Figure 7. Effect of Bromination Time on Extent of Bromination

Acidity, 0.75 *N*
Temperature, 25° C

methanes, and higher polymers, much less mixtures of these compounds. However, knowing which compounds brominate normally, and which brominate abnormally, and how various bromination conditions affect the results, it should be possible to determine accurately a large number of compounds by modifying the procedure where necessary. Further research is needed before bromination can be used for the quantitative analysis of the complex mixtures which result from the reaction of phenols with formaldehyde.

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Determination of Calcium in Magnesite and Fused Magnesia

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A method for the determination of calcium oxide in materials containing a high magnesium oxide content is proposed, whereby a separation of the magnesium from the lime is first effected. This procedure is the reverse of the usual technique employed. Mannite is used to prevent precipitation of the calcium during precipitation of the magnesium as hydroxide. The method is direct, requiring fewer preliminary separations than the existing methods. Quantitative recovery of the lime is assured. Spectrographic examination of a number of the precipitates of lime showed them to be of analytical purity, free from contamination by other impurities in the samples.

EXTREMELY small amounts of calcium, especially in the presence of much magnesium, as in magnesites and fused magnesia, cannot be determined satisfactorily by direct precipitation as oxalate (*3*). In material of this type, the usual procedure is to precipitate the calcium first as sulfate, then as oxalate, and to treat the residual sulfates so that a saturated solution of magnesium sulfate is formed. To this are added mixtures of ethyl alcohol, methyl alcohol, and sulfuric acid, in which the magnesium sulfate is appreciably soluble, while the insoluble calcium sulfate is removed by filtration.

While the calcium sulfate is purported to be wholly insoluble, careful study shows that results obtained by these procedures are usually somewhat low. The precipitation as sulfate is successful only in the absence of alkali metals, the sulfates of which are for the most part difficultly soluble in alcohol. This, of course, makes this method of separation entirely unsuitable for substances which must be first attacked by an alkali fusion—e.g., some fused and high-temperature fired periclase in which recrystallization has occurred.

In other methods for the separation of small amounts of lime from large amounts of magnesium, such as may occur in magnesium alloys (*2*, *5*), a sugar solution is used to dissolve the calcium hydroxide, leaving the magnesium hydroxide almost completely precipitated in strong alkali solution, from which the calcium may be separated by filtration.

The alloys, however, are relatively free of the impurities usually found in appreciable amounts in magnesite.

During work in this laboratory involving many thousands of boron analyses, solutions containing a polyhydric alcohol, in this particular case mannite, were titrated to an approximate pH of 11. Although in many cases rather large quantities of calcium were present as the chloride, no calcium hydroxide was precipitated. If, however, these solutions contained magnesium chloride, an appreciable precipitate of magnesium hydroxide occurred, rendering the boron analyses useless and making removal of the magnesium ion obligatory.

These phenomena suggested a possible new means of separating calcium from appreciable magnesium, providing conditions could be found which would yield a quantitative separation of the calcium from either all or a major portion of the magnesium.

EXPERIMENTAL

Samples of very high-grade, specially prepared magnesium oxide obtained from the high-temperature calcination of magnesium hydroxide were used in the preliminary study. This material was supplied by the J. T. Baker Chemical Company. Both chemical and spectrographic analysis showed that various shipments of this material contained from 0.04 to 0.08% calcium oxide.

Two-gram samples of this magnesium oxide were dissolved in hydrochloric acid and various amounts of reagent grade mannite from the Hercules Powder Company were added to the acid solution contained in 500-ml. volumetric flasks. The solution was then made alkaline with carbonate-free sodium hydroxide, diluted to 500 ml., mixed, and allowed to stand overnight. Examination of the clear supernatant liquid showed that from 95 to 99% of the magnesium had been removed.

The next step consisted of adding various amounts of a chloride solution prepared from calcium oxide purified by triple precipitation as oxalate after preliminary separation of the interfering elements. The additions of calcium ranged from 2 to 20 mg., calculated as oxide per 100 ml. of solution contained in the volumetric flask. After treatment of this solution with mannite and sodium hydroxide, as described in the general procedure, the calcium was separated from the clear supernatant liquid as oxalate by double precipitation. The recoveries obtained were excellent (Table I).

Following these tests, the sample of calcined magnesite No. 104 issued by the National Bureau of Standards, and a series of magnesite samples from various sources were analyzed by the method described. The results in Table I indicate excellent recoveries of the lime with accuracy equal to, or better than, some of the present generally accepted methods.

GENERAL PROCEDURE

Weigh out 2-gram samples of the magnesite or fused magnesia into 100-cc. porcelain casseroles, add 35 to 40 cc. of 1 to 1 hydrochloric acid, and warm until solution is effected. In the event that the material is not entirely soluble in the dilute hydrochloric acid, place the 2-gram sample in a 20-cc. platinum crucible and intimately mix with it about 3 to 5 grams of sodium carbonate. Place the crucible in a muffle furnace, or over a Fisher burner, sinter for about 30 minutes at 900° to 1000° C., cool, and dissolve in the casserole with 50 to 60 cc. of 1 to 1 hydrochloric acid.

After solution has been brought about by either method, evaporate the solution to dryness on a steam bath and bake the residue in a drying oven for one hour at 120° C. Add 5 cc. of concentrated

[1] Present address, Norton Co., Chippawa, Ontario.

Table I. Gravimetric Determination of Calcium in the Presence of Large Amounts of Magnesium

Substance	CaO Present	CaO Found by Usual Alcohol Method	Error, CaO	CaO Found by Proposed Method	Error, CaO
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*
MgO + CaO[a]	4.0	3.7	−0.3	3.9	−0.1
	8.0	7.8	−0.2	8.1	+0.1
	12.5	12.1	−0.4	12.4	−0.1
	16.0	15.6	−0.4	16.0	0.0
	20.0	19.8	−0.2	19.8	−0.2
	25.0	24.5	−0.5	25.0	0.0
	31.0	31.1	+0.1	31.1	+0.1
	36.0	35.5	−0.5	35.8	−0.2
	40.0	39.4	−0.6	40.1	+0.1
	40.0	39.5	−0.5	40.1	+0.1
India magnesite	8.5[b]	7.7	−0.8	8.9	+0.4
	9.1[b]	8.4	−0.7	9.2	+0.1
	9.7[b]	9.4	−0.3	10.0	+0.3
	9.9[b]	9.5	−0.4	9.9	0.0
Magnesite 104[c]	26.8	26.2	−0.6	27.4	+0.6
	26.8	26.6	−0.2	27.2	+0.4
	26.8	26.1	−0.7	27.4	+0.6
	26.8	26.1	−0.7	26.9	+0.1
	26.8	26.2	−0.6	27.3	+0.5
	26.8	26.4	−0.4	26.4	−0.4
	26.8	26.5	−0.3	26.2	−0.6
	26.8	26.0	−0.8	26.9	+0.1
	26.8	26.0	−0.8	27.0	+0.2
	26.8	26.1	−0.7	27.1	+0.3
Sea-water magnesite	18.0[b]	17.5	−0.5	17.9	−0.1

[a] Pure calcium oxide additions to magnesium oxide.
[b] Averaged values reported by chemical laboratory of Norton Co., Chippawa.
[c] U. S. National Bureau of Standards burned magnesite sample 104.

hydrochloric acid to the baked residue and then about 50 to 75 cc. of hot water, and digest until salts are in solution. Filter off the silica, washing the precipitate 4 to 5 times with hot 1 to 4 hydrochloric acid wash and finally with hot water. The silica content of the sample may be determined by the usual ignition and treatment of the precipitate with hydrofluoric acid, although very accurate determinations require two dehydrations. If the precipitated silica has been properly washed, no calcium will be found in the residue after hydrofluoric acid treatment.

To the cooled filtrate contained in a 500-cc. volumetric flask add 15 to 25 grams of mannite. When solution of the mannite has been effected, add 6 drops of phenolphthalein indicator and a solution of 9.0 *N* carbonate-free sodium hydroxide dropwise until the pink color of the indicator appears. The sodium hydroxide used must be carbonate-free because of the possibility of forming calcium carbonate, which is insoluble in the polyhydric alcohol solution and would lead to low lime results. Now add an excess of 5 cc. of the sodium hydroxide solution by dropwise additions while the solution in the flask is agitated to ensure prompt mixing of the two solutions. Cool the flasks to room temperature, dilute to 500 cc. with distilled water, shake the flask well, and allow to stand for 6 to 8 hours, or overnight. Pour the solution through a loose, dry, filter paper until a 200-cc. aliquot can be obtained. The aliquot may be removed by means of a pipet from the clear supernatant liquid in the flask, although in many cases this method is not satisfactory.

To the aliquots contained in 400-cc. beakers add 5 drops of methyl red indicator and acidify with hydrochloric acid, adding 5 cc. in excess. An addition of 5 grams of ammonium chloride at this point is desirable should, for some reason, an undue amount of magnesium remain in the solution. To the boiling solution add 0.5 gram of ammonium oxalate and, when dissolved, add ammonium hydroxide until the solution is neutral. Digest on a steam bath until the calcium oxalate precipitate is well formed. Remove, add 2 cc. of ammonium hydroxide dropwise, and allow precipitate to stand for 4 hours. Filter the solution through a No. 40 Whatman paper and wash with a cold 1% solution of ammonium oxalate.

Dissolve the washed precipitate through the paper with 50 cc. of hot 1 to 2 hydrochloric acid and reprecipitate, filter, and wash the calcium exactly as described for the initial precipitation. Convert the calcium oxalate to the oxide by ignition at 1100° to 1200° C. in tared platinum crucibles to constant weight, observing all of the usual precautions necessary for the weighing of this hygroscopic precipitate (*4*).

The 200-cc. aliquot taken for this analysis represents an 0.8-gram sample. The percentage of calcium oxide in the sample may be calculated as follows:

$$\frac{\text{Weight of precipitate} \times 100}{0.8} = \% \text{ CaO}$$

The authors prefer weighing the lime as oxide as described, but there is no objection to using the volumetric oxalate-permanganate titration to obtain the lime values. The described procedure is less subject to errors than the volumetric method.

DISCUSSION OF RESULTS

In this investigation, samples of magnesium oxide with various additions of pure calcium oxide in amounts equivalent to samples containing from 0.5 to 5.0% were analyzed by the above procedure.

In addition, ten separate and individual analyses of the sample of calcined magnesite No. 104, issued by the Bureau of Standards, four samples of India magnesite, and one sample of magnesite from sea water were analyzed by the same method. These latter samples were then analyzed by precipitation of the calcium as sulfate and then as oxalate after solution of the sulfate by the mixed alcohols.

The results of these investigations, which are shown in Table I, indicate that the values obtained by this new procedure are accurate and reliable. In general, as expected, the results are slightly higher than those found by the so-called alcohol method, probably because of the slight solubility of the calcium sulfate in the alcohol mixture.

The number of milligrams of calcium oxide present in the Bureau of Standards burned magnesite sample 104 is taken from the averaged certificate value of 3.35% calcium oxide (*1*). Four of the eight cooperating analysts reported higher values for lime than the averaged value. If the value obtained by the Bureau of Standards analyst were substituted for the value shown in the table, the calcium oxide present would be 27.0 mg. instead of 26.8 mg. Substitution of this value would show greater deviations for the analyses obtained by the alcohol, while the results obtained by the procedure proposed herein would show better agreement in eight of the ten analyses. While there is no justification for assuming that this value is better than the average reported value, the results obtained by the proposed method are in very close agreement with those reported by excellent analysts using the existing procedures.

The aluminum oxide content of standard sample 104 is given in the certificate as 0.84%. One might expect the aluminum to be a possible source of error in this method. Because of the solubility of the aluminum hydroxide in the sodium hydroxide solution, it might later be expected to coprecipitate partially or completely with the calcium. No trouble from this source was encountered.

A number of the calcium oxide precipitates obtained by the use of the proposed method were examined spectrographically for purity. In these examinations the spectra obtained using a large Bausch & Lomb Littrow type prism spectrograph were inspected critically. Only the usual spectrographic traces of impurities which are always detectable in the most precise chemical separations were present. The precipitates of calcium oxide obtained were judged, therefore, to be free from any coprecipitated constituents of the sample.

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A DISSERTATION submitted in partial fulfillment of the requirements for the degree of master of science to the faculty of the Graduate School of Niagara University.

Effect of Uniform versus Intermittent Product Withdrawal from Distillation Columns

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has long been tacitly assumed that the best way to operate high-ficiency distillation columns is with as nearly continuous a product-ithdrawal rate as possible. The relative effectiveness of intermit-nt vs. continuous product withdrawal for the separation of α- and -pinene was tested with a heligrid-packed, a helices-packed, and a auze-plate column. Especially with the heligrid-packed column istinctly better separation was obtained by product withdrawal nce every hour than once every 30 seconds.

RACTIONAL distillation is one of the most important laboratory tools in the field of hydrocarbon research. It ould be impossible to enumerate here all the publications deal-ig with laboratory and commercial fractionating columns. pecial mention should be made, however, of the excellent review f the literature on the construction, testing, and operation of aboratory fractionating columns made by Ward (*9*) and of two ore recent papers on distillation practices and methods by thmer (*4*) and Podbielniak (*8*). Despite these numerous pub-cations and the real progress made, usually empirically, in the evelopment of efficient laboratory fractionating columns, rela-vely little has been published on the practical operation of such olumns. There remains to be established a scientific basis for ertain operational techniques now in common use, such as the reflooding technique, which have been developed through prac-ical experience. That many differences of opinion exist con-erning the preferred mode of operation, even for major elements, s revealed by the following excerpts from Ward's review (*9*):

The usual distillation practice is to keep the distillation rate onstant. . . .Some operators, however, increase the rate when ollecting a pure component and decrease the rate between com-onents. . .Most investigators use the term "reflux ratio" to esignate the quantity of reflux per unit weight of product. . . IcMillan's results show higher efficiency with medium rather han high or low reflux ratio. Calin-aert and Huggins, with a coke-packed olumn, found the efficiency was pro-ortional to the reflux ratio at constant apor velocity. . .The usual practice s to maintain a constant reflux ratio; owever, some investigators decrease he reflux while collecting pure com-ounds and increase it at the critical oints between compounds. . . Judging rom their results, Hill and Ferris con-luded the peak efficiency of a packed olumn was at a rate just below flood-ng. Podbielniak found maximum ef-iciency when the packing is barely et. This is verified by Bruun and chicktanz, whose results show effici-ncy decreased materially near the ooding point.

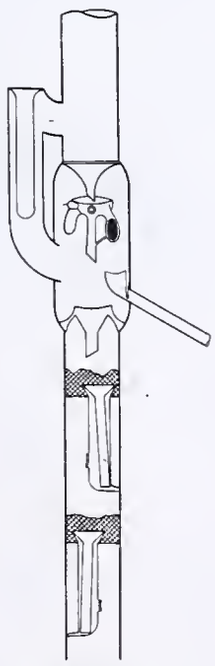

Figure 1. Reflux Controller and Top of Gauze Plate Column

Podbielniak (*8*) pointed out the ossibility that intermittent product ithdrawal might be superior to uni-orm product withdrawal:

The measurement and control of eflux ratio for high efficiency columns re most important in obtaining best ossible separation with a column in a iven time of distillation. . . It has long een tacitly assumed that the best way o operate any column is with as earl continuous a product-with-drawal rate as possible. . .However, in operating the high-efficiency column and packing here described, on close-boiling binary test mixtures at high reflux ratios, very much better separations have been obtained by holding the column at total reflux for periods of 30 minutes or more, and then withdrawing a quota of product almost instantaneously, again holding the column at total reflux, and so on, than by withdrawing the product continuously at the same average rate as in the intermittent operation. . .This unexpected advantage of intermittent product withdrawal, so outstanding as to merit the nickname "the free-wheeling effect" in the author's laboratory, still is without theoretical explanation.

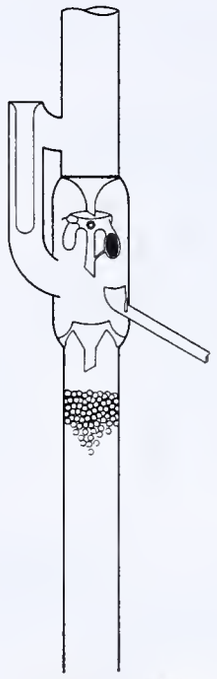

Figure 2. Reflux Controller and Top of Helices-Packed Column

In the laboratory of the Naval Stores Research Division complex mixtures of terpene hydrocarbons are often carefully fractionated into individual pure components. For this purpose, three different types of columns are available: a vacuum-jacketed Podbielniak type, 1 inch (2.5 cm.) in diameter, containing 4 feet of packing; an insulated, heat-compensated column, 1 inch in diameter, packed with $^3/_{16}$ inch Fenske-type glass helices for 7 feet; and a Palkin type (*5*), containing 45 individual gauze plates 1 inch in diameter, approximately 2 inches apart. Distillations are generally carried out at reduced pressure (usually at 20.0 mm. of mercury) to minimize thermal isomerization and at high reflux ratio to ensure maximum purity of product. Mixtures rich in α-pinene (boiling point at 20 mm. = 52.2° C.) and β-pinene (boiling point at 20 mm. = 59.7° C.) are frequently distilled. It was thought worth while, therefore, to investigate the utility of the "free-wheeling effect" in these distillations, especially since mixtures of pure α- and β-pinene can be analyzed conveniently and accurately simply by measurement of the optical rotation, Biot's relationship holding closely for these terpenes (*3*). Accordingly, two series of distillations were planned to test this free-wheeling effect.

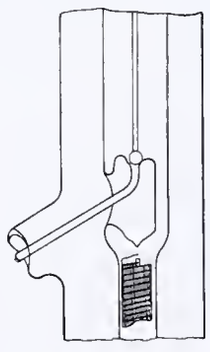

Figure 3. Reflux Controller and Top of Heligrid Column

EXPERIMENTAL PROCEDURE

In the first series of distillations each of the three columns was operated so that the product withdrawal, or take-off, was "uniform"; in the second series, so that the withdrawal was "intermittent". It was planned that in so far as possible the operation of all the columns in a series would be alike, thus permitting evaluation of the response of the various columns both with respect to each other and with respect to the manner of product withdrawal.

All three columns were operated simultaneously from a single control panel and "column line" manifold maintained at 20.0

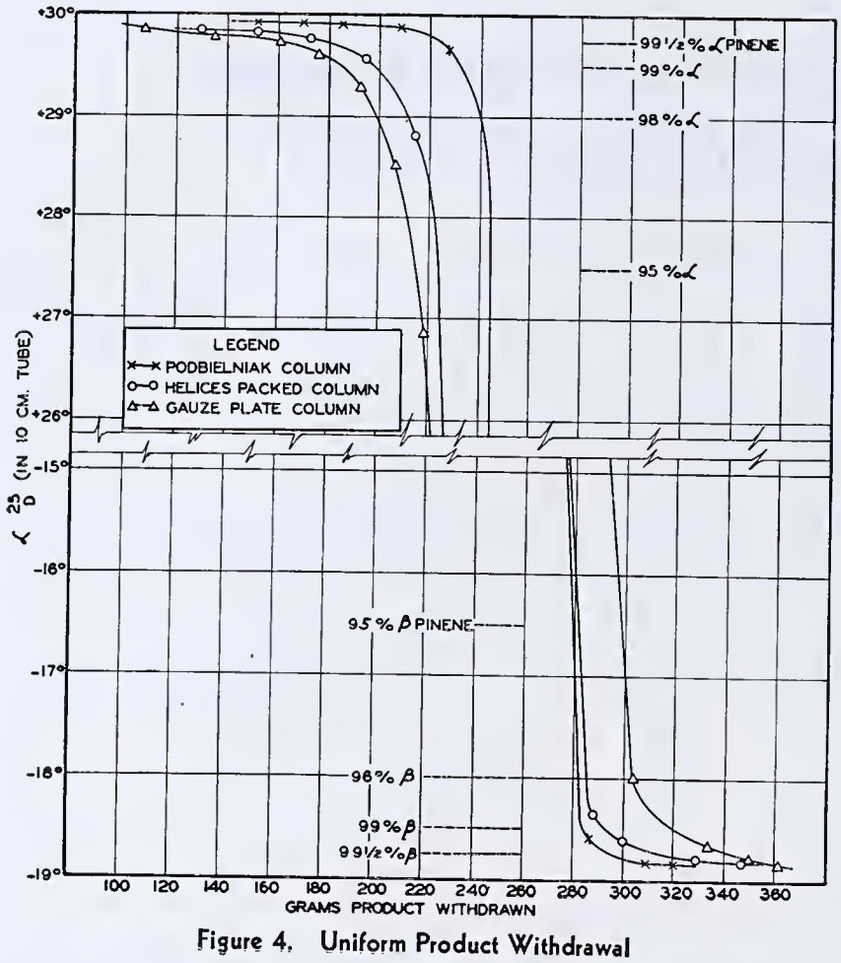

Figure 4. Uniform Product Withdrawal

mm. of mercury pressure by means of a modified Palkin-type dual pressure control assembly system (*6*). Throughput was maintained at 300 cc. per hour (at the head) by heating the still pots electrically. Current was supplied to Variacs through constant-voltage transformers and the Variacs were adjusted manually from time to time to maintain the desired throughput. Active ebullition was maintained by means of an electrically heated boiling promoter (*7*). Product take-off was maintained at 3 cc. per hour by suitable electrically operated timing devices. Each still pot was charged with 500 grams of a stock mixture of 50% α-pinene and 50% β-pinene (by weight), and distillation was continued without interruption until the β-pinene plateau had been reached or until reflux at the head ceased. Each distillation lasted about a week, and fractions were cut at 6- or 8-hour intervals.

Although it was desired to condition and operate all three columns as similarly as possible, certain dissimilarities could not be avoided, owing to the differences in construction of the three columns. For example, the Podbielniak column, being vacuum-jacketed, required no heat compensation for the jacket, whereas the heat loss from both the helices-packed and the gauze-plate columns was partially compensated by four thermostatically controlled, electrically heated resistance units in each column. In order to minimize possible differences arising from different manipulation of the thermostats, they were preset, so that each column would be losing a slight amount of heat at the boiling point of α-pinene and were left unchanged throughout the distillations. To obviate the possibility of differences in efficiency arising from any difference in degree of thoroughness of preflooding none of the columns was flooded.

Each column was allowed to reflux for approximately 5 hours before product withdrawal was begun. In the case of the helices-packed column and the gauze-plate column, each fitted with a modified head of the type described by Carter and Johnson (*2*) (Figures 1 and 2) "uniform" withdrawal was arranged for by use of a electric time switch operating on a 30-second cycle an set at 1%. In the case of the Podbielniak column (Fi ure 3) since the smallest portion of product that coul be withdrawn reliably was about 0.2 cc., the "uniform withdrawal was arranged for by use of an electric tim switch operating on a 5-minute cycle and set at 0.13% This gave a take-off of 3.0 cc. per hour when the reflu was 300 cc. per hour. In the case of the "intermit tent" withdrawal, all the columns had a product with drawal period of 36 seconds followed by a shut-in perio of 59 minutes and 24 seconds. This could be arrange for by use of a time switch operating on a 1-hour cycl and set at 1%. The optical rotation of each fractio (about 20 cc.) was measured and then plotted agains grams of product withdrawal from each column.

RESULTS AND DISCUSSION

Figure 4 shows such plots for some of the intermedi ate fractions obtained by uniform product withdrawa for all three columns operated simultaneously. Figure illustrates similar plots obtained using intermittent prod uct withdrawal. These plots permit a direct compari son of the effectiveness of all three columns, as well a an evaluation of the effect of uniform versus intermitten product withdrawal. [The performance of a colum measured in theoretical plates at atmospheric pressur cannot be interpreted as the plateage of that colum operating under vacuum. Thus Byron, Bowman, an Coull (*1*) point out that "a 50-plate column (at atmos pheric pressure) would realize only half a plate at 7.(mm. of mercury".] The fractions with the highest rota tion, for both α- and β-pinene, were obtained from the Podbielniak column. Table I shows for all six distil lations the size in grams of the intermediate portions between α- and β-pinene at various levels of purity—i.e., 95, 98 99, and 99.5%. Also shown in this table is the difference in the

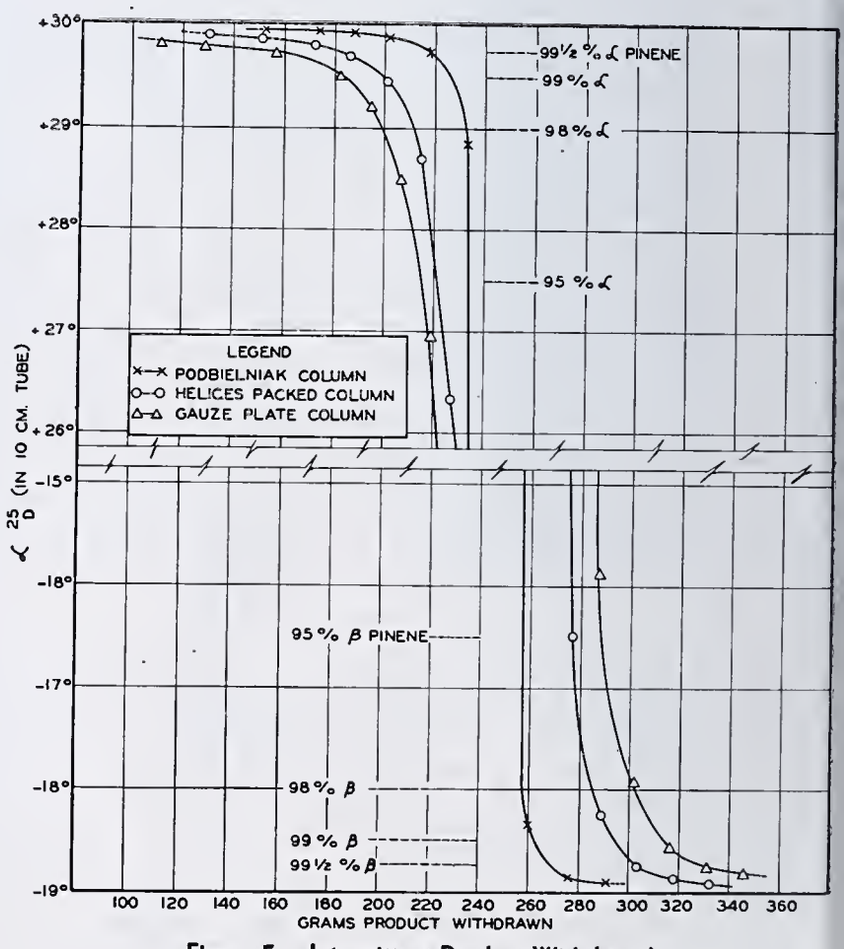

Figure 5. Intermittent Product Withdrawal

Table I. Comparison of Uniform and Intermittent Product Withdrawal

	Grams of Product between α- and β-Pinene of Indicated Purity			
	99.5%	99%	98%	95%
Podbielniak column				
Uniform	72	55	43	35
Intermittent	52	37	27	23
Decrease	20	18	16	12
Helices-packed column				
Uniform	138	97	74	58
Intermittent	121	95	73	56
Decrease	17	2	1	2
Gauze-plate column				
Uniform	184	138	105	87
Intermittent	171	130	103	72
Decrease	13	8	2	15

quantity of material of intermediate purity obtained when operating under uniform as against intermittent withdrawal. Of the three columns tested the Podbielniak column, when operated under the conditions of this test, made the best showing; the gauze-plate column, the poorest. Furthermore, for the Podbielniak column, when intermittent rather than uniform product withdrawal was used, the improvement was very marked and consistent, showing a decrease in size of intermediate fractions of approximate 35% at all levels of purity. Although some improvement was also apparent in the operation of the other two columns, this improvement in several cases was within the limits of possible experimental error.

It may, therefore, be concluded that when operating high-efficiency columns at high reflux ratios it is not necessary to use as nearly continuous product withdrawal as possible. In fact, when a reflux ratio of 100 to 1 is used, better separations are obtained by product withdrawal once every hour than once every 30 seconds.

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Determination of 1-Trichloro-2,2-bis(p-chlorophenyl)ethane in DDT Dusts and Oil Solutions

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A method is described for the determination of 1-trichloro-2,2-bis(p-chlorophenyl)ethane (*p,p'*-DDT) in commercial dusts and oil solutions. The method is based upon the dehydrochlorination of the sample at 25.0° ± 0.1° C. in 95% ethyl alcohol solution for dust mixtures, and in kerosene solution for oil solutions, for 15 minutes, using 20 ml. of *N* ethanolic sodium hydroxide. Comparative results are given for the crystallization method and the method described. Possible sources of error are discussed.

THE insecticidal value of an economic poison containing DDT is believed primarily due to its content of the para-para' isomer, 1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (*p,p'*-DDT); and in enforcement of the Agricultural Code of the State of California pertaining to labeling and sale of agricultural chemicals, it is necessary to determine the percentage of this compound in commercial dusts and sprays. Several useful methods have been developed (*6*, *7*, *8*, *13*, *15*) for determining or detecting total DDT, but they are not applicable to the quantitative estimation of the individual principal isomers occurring in commercial DDT. The crystallization method of Cristol, Hayes, and Haller (*5*) has proved most valuable for the determination of *p,p'*-DDT in the technical product and straight DDT dust formulations, but it is not applicable to oil solutions, such as fly sprays, or dusts containing pyrethrum powder or concentrates and is time-consuming when applied to low percentage dust mixtures. The complex mixtures making up modern commercial insecticides cause serious interference in most colorimetric methods (*1*, *3*, *10*).

Cristol (*4*) has suggested that the difference in reaction rates of *p,p'*-DDT and *o,p'*-DDT to dehydrochlorination might be used for determination of the para-para' isomer in technical DDT. This reaction was investigated by the author, and procedures were developed applicable to most commercial DDT dusts and sprays. [Since the submission of this paper an article on the determination of *p,p'*-DDT in technical DDT has been published (*12*).]

EXPERIMENTAL

A number of commercially available technical and purified grade DDT samples of known *p,p'*-DDT content, as determined by the Cristol, Hayes, and Haller crystallization method (*4*), were dehydrochlorinated under strict conditions of temperature and concentration.

A 1.000-gram sample was dissolved in 75.0 ml. of 95% ethyl alcohol, and reacted 15 minutes with 20.00 ml. of *N* ethanolic sodium hydroxide at 25.0° ± 0.1° C. A graph of the per cent chloride ion formed, as ordinate, and per cent *p,p'*-DDT of the samples as abscissa, gave a straight line, which could be expressed by the formula:

$$(\%\ \text{chloride} \times 11) - 9.30 = \%\ p,p'\text{-DDT}$$

A 0.500-gram sample was dissolved in kerosene, made up to 100.0 ml. with kerosene, and reacted 15 minutes with 20.00 ml. of *N* ethanolic sodium hydroxide at 25.0° ± 0.1° C., while being stirred at 700 to 800 r.p.m. A graph of the per cent chloride ion formed and per cent *p,p'*-DDT of the samples gave a straight line, expressible by the formula:

$$(\%\ \text{chloride} \times 23) - 16.90\% = \%\ p,p'\text{-DDT}$$

REAGENTS AND APPARATUS

Ethyl alcohol, commercial 190-proof, 95% by volume.

Ethanolic sodium hydroxide, 1.000 *N*. Dissolve 41 to 42 grams of C.P. sodium hydroxide pellets in about 350 ml. of ethyl alcohol with the aid of heat, cool, and filter into a 1-liter volumetric flask. Add alcohol almost to mark, and mix. Standard-

ize against 0.5 or 0.2 *N* hydrochloric acid, using methyl orange indicator.

Ethanolic potassium or sodium hydroxide, approximately 1 *N*. Dissolve 56 grams of C.P. potassium hydroxide or 40 grams of C.P. sodium hydroxide in about 250 ml. of ethyl alcohol, filter, and make to 1 liter with ethyl alcohol.

Nitric acid, 1 to 1, 1 volume of C.P. concentrated nitric acid plus 1 volume of distilled water.

Nitric acid, 1 to 3, 1 volume of C.P. concentrated nitric acid plus 3 volumes of distilled water.

Silver nitrate 0.1 *N*, 16.989 grams of silver nitrate per liter of solution. Standardize by precipitating and weighing as silver chloride.

Ammonium thiocyanate, 0.1 *N*, 7.611 grams of ammonium thiocyanate per liter solution. Standardize against the 0.1 *N* silver nitrate.

Ferric alum indicator, 10%, 10 grams of C.P. ferric ammonium sulfate dissolved in a minimum of distilled water. Filter, decolorize with concentrated nitric acid, and dilute to 100 ml.

Nitrobenzene, purified grade mononitrobenzene.

Acetone, C.P. reagent grade.

Kerosene, a good quality water-white kerosene or deodorized kerosene.

Constant-temperature bath, capable of maintaining a temperature of 25.0 ± 0.1° C., and large enough to hold two 250-ml. flasks.

Electric stirrer, fitted with a 18- to 20-mm., two-blade glass stirrer, speed adjustable to 700 to 800 r.p.m.

Interval timer, "minute minder", or clock with sweep-second hand.

Parr bomb.

PROCEDURE FOR DRY MATERIALS

Technical and Purified DDT (96 to 100% from total chlorine). A 1.000-gram sample of the material is placed in a 250-ml. ⅁ Erlenmeyer flask, 75.0 ml. of 95% ethyl alcohol are added, and the flask is heated under reflux until the sample is dissolved. The solution is cooled and maintained at 25.0° ± 0.1° C. in a thermostatically controlled bath, 20.00 ml. of 1.000 *N* ethanolic sodium hydroxide, also maintained at 25.0° ± 0.1° C., are added, and the contents are mixed by rotating. The flask is rotated about every 5 minutes. At the end of 15 minutes the reaction is quickly stopped by the addition of 15 ml. of nitric acid, 1 to 3. The flask is removed from the bath, and 30.00 ml. of 0.1 *N* silver nitrate, 3 ml. of 10% ferric alum indicator, and 5 ml. of nitrobenzene are added. The flask is stoppered and shaken a few seconds to coagulate the precipitate and coat the particles of silver chloride (*2*). The stopper is washed down and the excess silver nitrate is titrated with 0.1 *N* ammonium thiocyanate. The per cent chloride ion formed is calculated:

$$\text{Ml. of 0.1 } N \text{ } AgNO_3 \times 0.3546 = \% \text{ chloride}$$

The per cent of *p,p'*-DDT is calculated from the per cent chloride:

$$(\% \text{ chloride} \times 11) - 9.30 = \% \text{ } p,p'\text{-DDT in sample}$$

DDT Dust Mixtures Not Containing Sulfur or Organic Thiocyanates. *Total DDT.* Dust mixtures containing unknown amounts of DDT must first be analyzed for percentage of total DDT.

Samples containing 5 to 80% of total DDT can be ignited in a Parr peroxide bomb, or refluxed with metallic sodium and isopropyl alcohol (*14*) and total DDT calculated from total chlorine:

$$\frac{\text{Ml. of 0.1 } N \text{ } AgNO_3 \times 0.7092}{\text{sample weight}} = \% \text{ total DDT}$$

Samples containing 1 to 5% of DDT are most conveniently analyzed by the Gunther method (*7*), using 5 to 10 grams of sample which is refluxed 15 minutes with 30 ml. of *N* ethanolic potassium or sodium hydroxide. The flask is cooled and the solution acidified with dilute nitric acid. An excess of 0.1 *N* silver nitrate, 3 ml. of ferric alum solution, and 5 ml. of nitrobenzene are added and the flask is shaken to coagulate and cover the silver chloride particles. The excess 0.1 *N* silver nitrate is titrated with 0.1 *N* ammonium thiocyanate:

$$\frac{\text{Ml. of 0.1 } N \text{ } AgNO_3 \times 3.546}{\text{sample weight}} = \% \text{ total DDT}$$

p,p'-DDT. The per cent total DDT divided into 100 yields the sample weight in grams equivalent to 1.000 gram of total DDT. This sample weight is run through the same procedure used for technical and purified DDT. Dusts of high DDT content can usually be titrated for the chloride produced by the dehydrochlorination reaction without first filtering.

Add 30.00 ml. of 0.1 *N* silver nitrate, 3 ml. of ferric alum indicator, and 5 ml. of nitrobenzene, shake, and titrate with 0.1 *N* ammonium thiocyanate. Calculate the per cent chloride formed and from this the percentage of *p,p'*-DDT.

Example. If total DDT is 5.68%, then 100/5.68 = 17.60 grams equivalent to 1.000 gram of total DDT subject to dehydrochlorination.

30.00 ml. of 0.1 *N* $AgNO_3$ − 9.60 ml. of 0.1 *N* NH_4SCN = 20.40 ml. of 0.1 *N* $AgNO_3$ used.
20.40 × 0.3546 = 7.23% chloride ion formed during dehydrochlorination.
(7.23 × 11) − 9.30 = 70.23% of the total DDT is *p,p'*-DDT.
5.68% total DDT in sample multiplied by 0.7023 = 3.99% *p,p'*-DDT in sample.

The inert fillers usually do not obscure the end point. Dusts of low DDT content containing a large amount of insoluble filler must usually be filtered following the dehydrochlorination reaction, carefully washing all soluble chloride from the filler. Suction should not be used because of the possibility of losing chloride from the acid solution.

DDT Dust Mixtures Containing Sulfur. *Total DDT.* Samples containing 10% or more total DDT are first analyzed for total DDT by using a Parr bomb. Low percentage DDT-sulfur mixtures containing 1 to 10% DDT and 50 to 90% sulfur can be analyzed by the Gunther method (*7*) if modified as follows:

A sample weight large enough to give an accurate titration—i.e., 5 grams of a dust containing 5% total DDT—is refluxed 10 minutes with 25 to 40 ml. of acetone. The flask is cooled in an ice bath or refrigerator for about an hour to reduce the solubility of sulfur in acetone. The sulfur is filtered off, using a small Büchner funnel and suction, and washed twice with cold acetone. The acetone in the filtrate, containing the DDT, is boiled off on a steam bath with the aid of a stream of air, being heated only long enough to remove the acetone. The DDT residue is refluxed with 30 ml. of *N* ethanolic potassium or sodium hydroxide for 15 minutes and cooled. A measured excess of 0.1 *N* silver nitrate, 50 ml. of distilled water, and a few Carborundum chips are added to the flask, and the flask is boiled gently on a hot plate until most of the alcohol is boiled off. The flask is cooled slightly, 20 ml. of 1 to 1 nitric acid are added, and the solution is boiled until all silver sulfide is decomposed. Care must be taken to prevent loss through bumping. The flask is cooled, 3 ml. of ferric alum solution and 5 ml. of nitrobenzene are added, the flask is stoppered and shaken, and the excess silver nitrate is titrated. The per cent total DDT is calculated using the factor 3.546.

p,p'-DDT. A sample weight equivalent to 1.000 gram of total DDT, calculated from the total DDT percentage, is refluxed with 40 ml. of acetone for 10 minutes, then cooled in an ice bath or refrigerator. The sulfur is filtered off by suction, and the filtrate is caught in a 250-ml. ⅁ Erlenmeyer flask. The filter is washed twice with cold acetone. The acetone is boiled off on a steam bath using an air stream (avoid heating after the removal of acetone). The DDT residue is then refluxed with 75.0 ml. of 95% ethyl alcohol, cooled, and dehydrochlorinated at 25.0° ± 0.1° C. for 15 minutes with 20.00 ml. of 1.000 *N* ethanolic sodium hydroxide. The reaction is stopped by adding 15 ml. of 1 to 3 nitric acid. The flask is removed from the bath, 30.00 ml. of 0.1 *N* silver nitrate, 50 ml. of distilled water, and Carborundum chips are added to the flask, and the flask is heated on a hot plate until most of the alcohol has boiled off. Then 20 ml. of 1 to 1 nitric acid are added and the solution is boiled until all silver sulfide is decomposed. The flask is cooled, and after addition of ferric alum solution, and nitrobenzene and shaking, the excess 0.1 *N* silver nitrate is titrated. The percentage chloride and *p,p'*-DDT are calculated as in DDT dust mixtures not containing sulfur.

DDT Dust Mixtures Containing Organic Thiocyanates. *Total DDT.* Total DDT in the sample can be determined from total chloride by using a Parr bomb. The Gunther method (*7*) can be used on low percentage DDT samples if modified. Alkali hydroxides react with organic thiocyanates to form cyanides and cyanates (*9*), which react with silver nitrate, causing high results.

The sample is refluxed with ethanolic potassium or sodium hydroxide, cooled, and filtered into a separatory funnel; the filter is washed thoroughly. The filtrate is acidified slightly with dilute nitric acid and extracted twice with 25-ml. portions of petroleum ether to remove the dichloroethylene derivative of DDT. The subsequent boiling with nitric acid would yield chloride if the DDT olefin was not removed. The lower aqueous layer is drained into a 400- or 600-ml. beaker. The petroleum ether layers are washed twice with water, and the washings are added to the beaker. A measured excess of 0.1 *N* silver nitrate is added to the beaker, followed by 25 ml. of concentrated nitric acid and a few boiling chips. The beaker is covered with a watch glass, boiled down on a hot plate almost to dryness, and cooled slightly, 10 ml. more of nitric acid are added, and the contents are again boiled down almost to dryness. Silver cyanate and cyanide are decomposed by boiling nitric acid (*11*). The beaker is cooled and the contents are washed into a 250-ml. ⅋ Erlenmeyer flask. After the addition of ferric alum and nitrobenzene and shaking, the excess 0.1 *N* silver nitrate is titrated and the per cent total DDT calculated as described for mixtures not containing sulfur.

p,p'-DDT. Using a sample weight equivalent to 1.000 gram of total DDT, the sample is dehydrochlorinated with 20.00 ml. of 1.000 *N* ethanolic sodium hydroxide for 15 minutes at 25.0° ± 0.1° C., stopping the reaction with 15 ml. of 1 to 3 nitric acid. The contents of the reaction flask are filtered through a coarse paper into a separatory funnel. The use of suction is to be avoided because of possible chloride loss. The filter is washed with water sufficiently to remove soluble chloride. The combined filtrate and washings are extracted twice with petroleum ether to remove DDT olefin, and the lower layer is drained into a 600-ml. beaker. The petroleum ether layers are washed twice with water and the washings are added to the beaker. Then 30.00 ml. of 0.1 *N* silver nitrate, 25 ml. of concentrated nitric acid, and a few boiling chips are added. The beaker is covered with a watch glass and the solution boiled down almost to dryness on a hot plate. The beaker is cooled slightly, 10 ml. of nitric acid are added, and it is again boiled down. The beaker is cooled and the contents are washed into a ⅋ Erlenmeyer flask. Ferric alum and nitrobenzene are added, the flask is shaken, and the excess silver nitrate titrated. The per cent *p,p'*-DDT is calculated.

PROCEDURE FOR OIL SOLUTIONS

TOTAL DDT. The Gunther method (*7*) is the most convenient method for determining the percentage of total DDT in oil solutions.

A sample of the DDT oil solution, weighing 5 to 20 grams, is refluxed with 30 ml. of approximately *N* ethanolic potassium or sodium hydroxide for 15 minutes. The flask is cooled and acidified slightly with dilute nitric acid, and the contents are washed into a separatory funnel. The lower layer is drained into a 250-ml. ⅋ Erlenmeyer flask, and the upper layer is washed twice with water, the washings being added to the flask. A measured amount of 0.1 *N* silver nitrate, known to be an excess, is added to the flask along with 3 ml. of ferric alum solution and 5 ml. of nitrobenzene. The flask is stoppered and shaken, and the excess 0.1 *N* silver nitrate titrated with 0.1 *N* ammonium thiocyanate. Total DDT is calculated using the factor 3.546, as for DDT dust mixtures.

When organic thiocyanates are present, the method must be modified as follows:

The weighed sample is refluxed as described above, cooled, and transferred to a separatory funnel. It is then acidified with dilute nitric acid, and extracted twice with 25-ml. portions of petroleum ether to remove the dichloroethylene derivative of DDT. The lower layer is drained into a 600-ml. beaker. The oil layer is washed twice with water and the washings are added to the beaker. A measured excess of 0.1 *N* silver nitrate, 25 ml. of concentrated nitric acid, and a few boiling chips are added to the beaker. The beaker is covered with a watch glass, and boiled down almost to dryness on a hot plate. The beaker is cooled, and 10 ml. more nitric acid are added and the contents boiled down again. After cooling, the contents are washed into a 250-ml. ⅋ Erlenmeyer flask. On adding ferric alum and nitrobenzene and shaking, the excess silver nitrate is titrated with 0.1 *N* ammonium thiocyanate. Total DDT is calculated using the factor 3.546.

p,p'-DDT. A sample weight equivalent to 0.500 gram of total DDT, calculated from the per cent total DDT, is weighed into a 250-ml. Erlenmeyer flask. The volume of this weight of sample is calculated by dividing the sample by the density. This volume is subtracted from 100, giving the number of milliliters of kerosene to be added to the flask to make a total solution volume of 100.0 ml. The flask is placed in a thermostatically controlled bath and maintained at 25.0° ± 0.1° C. An 18- or 20-mm. glass, two-blade stirrer, is placed in the flask, so that the blades are just off the bottom of the flask. While the oil solution is stirred at 700 to 800 r.p.m., 20.00 ml. of 1.000 *N* ethanolic sodium hydroxide, also maintained at 25.0° ± 0.1° C., are added and the reaction is allowed to continue 15 minutes. The reaction is stopped by the addition of 15 ml. of 1 to 3 nitric acid. The stirrer is stopped and the contents of the flask are washed into a separatory funnel. The lower layer is drawn off into a 250 ml. ⅋ Erlenmeyer flask. The oil layer is washed twice with water and the washings are added to the flask. The per cent chloride formed is determined by adding 10.00 ml. of 0.1 *N* silver nitrate, 3 ml. of ferric alum solution, and 5 ml. of nitrobenzene, shaking, and titrating the excess silver nitrate with 0.1 *N* ammonium thiocyanate:

$$\frac{\text{Ml. of 0.1 } N \text{ } AgNO_3 \times 0.3546}{0.5} = \% \text{ chloride}$$

$$(\% \text{ chloride} \times 23) - 16.90 = \% \text{ } p,p'\text{-DDT in total DDT}$$

$$\frac{\% \text{ total DDT} \times \% \text{ } p,p'\text{-DDT}}{100} = \% \text{ } p,p'\text{-DDT in sample}$$

If organic thiocyanates are present, the above procedure is modified.

The sample and kerosene are dehydrochlorinated as described above, 20.00 ml. of 1.000 *N* ethanolic sodium hydroxide are added while stirring at 700 to 800 r.p.m., the reaction is allowed to proceed 15 minutes and then stopped by adding 15 ml. of 1 + 3 nitric acid. The reaction mixture is washed into a separatory funnel, and extracted twice with 25-ml. portions of petroleum ether. The lower layer is drained into a 600-ml. beaker. The oil layers are washed twice with water and the washings are added to the beaker. Then 10.00 ml. of 0.1 *N* silver nitrate, 25 ml. of concentrated nitric acid, and a few boiling chips are added to the beaker. The beaker is covered with a watch glass and the solution boiled down almost to dryness on a hot plate. The beaker is cooled, 10 ml. more of concentrated nitric acid are added and the solution is again boiled down. When cool, the contents are washed into a 250-ml. ⅋ Erlenmeyer flask. Ferric alum solution and nitrobenzene are added, the flask is shaken, and the excess 0.1 *N* silver nitrate is titrated with 0.1 *N* ammonium thiocyanate. The per cent chloride formed, and per cent *p,p'*-DDT are calculated as described for regular oil solutions of DDT.

DISCUSSION

Preliminary tests indicated that a sample weight of 1.000 gram of total DDT was about optimum for dry materials. Larger sample weights gave only slightly more chloride ion on dehydrochlorination, which was offset by an increase in interfering substances.

Tests were run to determine the effect of agitation during dehydrochlorination of DDT dust samples. Identical results were obtained when the reaction mixture was stirred continually at 700 r.p.m., or just mixed by rotating the flask three times during the 15-minute period.

Most DDT oil sprays contain at least 1% total DDT by weight. In order to keep the solution volume to a minimum, a sample weight equivalent to 0.500 gram of DDT was used. The addition of alcohol to the oil solution was found inadvisable because the alcohol dilutes the ethanolic sodium hydroxide, producing less chloride ion during dehydrochlorination.

Attempts to use 1.000 *N* butanolic sodium hydroxide in place of ethanolic sodium hydroxide for the dehydrochlorination of DDT in oil solutions were unsuccessful.

The Gunther method (*7*) gives slightly higher results for total DDT than total DDT calculated from total chlorine. Tests indicated that the percentage of *p,p'*-DDT, in samples containing 5% or less of DDT was closer to theoretical when total DDT was determined by the Gunther method (*7*) than when total DDT was calculated from total chlorine.

An accuracy of 2% *p,p'*-DDT can be expected for most dry DDT mixtures, and 4% *p,p'*-DDT oil sprays. Pyrethrins can

Table I. Determination of *p,p'*-DDT in Dust Mixtures of Known Composition

Sample No.	Composition	% *p,p'*-DDT In Sample	% *p,p'*-DDT Found	% Recovery
1	20% DDT (total) 80% talc	15.41	15.40 15.36	
			Av. 15.38	99.81
2	10% DDT (total) 90% talc	7.88	7.85 7.82	
			Av. 7.84	99.49
3	5% DDT (total) 50% sulfur 45% talc	3.85	3.91 3.90	
			Av. 3.91	101.56
4	5% DDT (total) 50% sulfur 45% talc	3.94	4.05 4.03	
			Av. 4.04	102.54
5	10% DDT (total) 85.25% talc 4.75% Lethane 60[a]	7.34	7.32 7.26	
			Av. 7.29	99.32
6	10% DDT (total) 88.8 % talc 0.2 % pyrethrins 1.0 % oil	7.63	7.22 7.21	
			Av. 7.22	94.63
7	10% DDT (total) 89.4 % talc 0.1 % pyrethrins 0.5 % oil	7.88	7.52 7.54	
			Av. 7.53	99.56

[a] Contains β-thiocyanoethyl esters of aliphatic fatty acids averaging 10 to 18 carbon atoms.

Table II. Comparative Analyses of Commercial DDT Samples

Sample No.	Per Cent *p,p'*-DDT Found: Crystallization method	Dehydrochlorination method	Difference
Technical and Purified Grade DDT Samples			
1	75.07	74.85	0.22
2	77.07	76.28	0.79
3	77.95	78.04	0.09
4	78.76	78.36	0.40
5	86.04	85.74	0.30
6	89.10	88.82	0.28
7	97.00	96.85	0.15
8	99.45	98.74	0.71
		Average difference	0.37
Commercial DDT Dust Mixtures (Not Containing Sulfur)			
45106[a]	5.36	5.44	0.08
44398	8.19	8.12	0.07
44239	13.22	12.95	0.27
44327	18.17	18.89	0.62
44889	37.34	37.47	0.13
43929	39.11	39.20	0.09
43923	39.63	38.82	0.81
		Average difference	0.30
Commercial DDT Dusts Containing 50% Sulfur			
45110	3.43	4.28	0.85
45072	3.83	4.03	0.20
45114	3.97	4.16	0.19
45111[b]	4.95	4.36	0.59
		Average difference	0.46

[a] Contains β,β'-dithiocyanodiethyl ether.
[b] Contains basic sulfates of copper and zinc and 2.10% sulfur.

cause an error of 5% *p,p'*-DDT in dust formulations. Duplicate results should agree within 0.5% *p,p'*-DDT.

The values of *p,p'*-DDT obtained by the method described are relative to the crystallization method as a standard. There is no method known to give the true values of *p,p'*-DDT in technical DDT, though the crystallization method gives results which are believed to be closest to the truth.

SOURCE OF ERROR

A source of error is the presence of water-soluble chlorides in some types of commercial DDT dusts. The presence of 0.1% water-soluble chloride will cause a positive error of 1.0% total DDT if the Gunther method (7) is used. This would lower the sample weight equivalent to 1.000 gram of total DDT and cause an error in *p,p'*-DDT content. Only four samples of the many analyzed were found to contain appreciable amounts of water-soluble chlorides. Their presence, once established, can usually

Table III. Determination of Known Amounts of *p,p'*-DDT in Oil Solutions

Sample No.	Composition	% *p,p'*-DDT In sample	% *p,p'*-DDT Found	% Recovery
1	Kerosene solution[a]	1.53	1.55	101.31
2	Spray oil solution[b]	4.23	4.45	105.20
3	Kerosene solution 0.1% pyrethrins	1.55	1.61	103.87
4	Kerosene solution 5% Lethane 60[c]	2.68	2.58	96.27

[a] Specific gravity 0.7940 at 20.0° C.
[b] Light-medium emulsible spray oil, specific gravity 0.8729 at 20.0° C.
[c] Contains β-thiocyanoethyl esters of aliphatic fatty acids averaging 10 to 18 carbon atoms.

Table IV. Effect of Time on Dehydrochlorination

Reaction Time *Min.*	Chloride Formed %
5	6.45
7	7.09
10	7.52
15	7.77
20	7.91

Table V. Effect of Stirring Speed on Dehydrochlorination of DDT in Oil Solutions

Stirring Speed *R.p.m.*	Chloride Formed %
500	3.79
700	4.03
800	4.04

be determined quantitatively and a correction made. If large amounts are found present it might be easier to extract the DDT with benzene and determine the total DDT in the benzene extract. Dust of unknown composition should be checked qualitatively for water-soluble chloride ions before beginning a quantitative determination of DDT.

A graph of Table IV will show that any error due to the measurement of time during the dehydrochlorination reaction will be less at 15 minutes than at any shorter period. This relationship holds for both DDT dusts and oil solutions.

In the analysis of DDT-sulfur mixtures it is imperative that all silver sulfide be decomposed before back-titrating with thiocyanate: otherwise an appreciable error will be introduced. A positive error in total DDT will cause a negative error in the *p,p'*-DDT percentage. If the error occurs only during the *p,p'*-DDT determination, the results will be high.

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Rapid Electrometric Determination of the Alkalinity of Sea Water

Using a Glass Electrode

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On the basis of glass electrode pH measurements of solutions to which known amounts of dilute hydrochloric acid have been added, "empirical coefficients" have been calculated, and the normality of a dilute solution of sodium bicarbonate containing a mixture of salts has been determined with a precision of about 1%. The operations are illustrated in connection with determination of the alkalinity of sea water. One measurement of pH yields the normality or alkalinity directly and a direct-reading pH instrument could be scaled to read normality, molarity, or alkalinity directly. The factors that affect the accuracy of the determination are listed and their magnitudes indicated. All steps are indicated that must be performed in developing a similar procedure for systems other than sea water. Industrial control determinations, and clinical investigations where large numbers of determinations are made, can be similarly standardized.

THE term "alkalinity" has been adopted by the International Association of Physical Oceanography (1939) as the standard designation to replace the terms "titratable base", "excess base", "titration alkalinity", and "buffer capacity" of sea water. It is defined as being the number of milliequivalents of hydrogen ion neutralized by 1 liter of sea water at 20° C. The alkalinity of sea water is primarily due to the presence of bicarbonates, though borates, phosphates, arsenites, and silicates are present in lesser amounts to form a complex buffered system with a pH of about 8. Information concerning the total concentration of salts of these weak acids as well as the available alkali cannot be acquired from a single pH measurement but must be determined otherwise.

The methods for the determination of alkalinity of sea water have been well summarized by Sverdrup, Johnson, and Fleming (*3*). Because of the variety of results obtained by the different methods, there is a definite need for a standard procedure yielding accurate and precise results which can be universally understood and compared. None of the methods involves any assumptions as to the nature of the buffers present in sea water, but all take into account the variation in salt content. The work of Mitchell and Rakestraw (*2*) was based upon a titration procedure and yielded excellent results. In the method of Thompson and Bonnar (*5*) an excess of standard acid was added to the sea-water sample. The amount of the acid that had been effectively neutralized by the sea water was readily determined by measuring the amount of excess acid colorimetrically, by comparison with a series of standard tubes. Though the method is convenient, the standard comparison tubes were difficult to prepare and rather unstable.

Some time ago the glass electrode was used at this laboratory by Thompson and Anderson (*4*) for making routine pH and alkalinity measurements of sea water. This eliminated some of the difficulties of the earlier methods, but new considerations arose. Further investigation of the use of the glass electrode by the present authors in determining the concentration of the excess acid has demonstrated the necessity of a knowledge of the relationship between the pH of the solution and the amount of acid added to sea water of various salinities. This involves the experimental determination of coefficients which are empirical, having significance only when applied to this determination with the specified conditions.

DETERMINATION OF EMPIRICAL COEFFICIENT

The empirical coefficients were determined from a series of titrations of natural and artificial sea-water samples. Sea water is alkaline to about the same extent as a 0.002 *N* sodium bicarbonate solution. One hundred milliliter samples were titrated with 0.01000 *N* hydrochloric acid until the pH was about 3.0. The equivalent point was taken as occurring with the greatest change in e.m.f. per unit volume of added acid, at a pH of about 4.5. The pH of the solution was measured at frequent intervals beyond the equivalence point. From the known amount of acid added and the experimentally determined alkalinity, it was possible to determine the excess acid present. The empirical coefficient, f_{H^+}, was calculated by dividing $C_{H^+}f_{H^+}$ obtained from the pH measurement of the solution by the concentration of excess strong acid, C_{H^+}, as shown under calculations. From this coefficient, C_{H^+} in other similar solutions can be calculated.

EXPERIMENTAL EQUIPMENT. The pH measurements were made with Leeds & Northrup glass electrodes No. 1199–12 and reference electrodes No. 1199-13. These were connected with a Leeds & Northrup vacuum tube amplifier to a Type K2 potentiometer.

EXPERIMENTAL PROCEDURE. The electrodes were standardized according to the method recommended by Dole (*1*) against a potassium acid phthalate solution, which was prepared from salt obtained from the National Bureau of Standards according to directions supplied by the bureau. An additional check was made as recommended by Dole against sodium borate. The $E°$ values were determined before each series of titrations, using Equation 1:

$$E° = E - \frac{2.3RT}{nF}\,\mathrm{pH} \qquad (1)$$

A concentrated synthetic sample of sea water having the following composition was prepared from carefully purified salts: NaCl, 0.4920 *M*; $MgCl_2$, 0.0646 *M*; Na_2SO_4, 0.0338 *M*; $CaCl_2$, 0.0138 *M*; KCl, 0.0123 *M*; $NaHCO_3$, 0.0026 *M*. Solutions for titration were prepared by dilution of this concentrated solution.

Eight samples of natural and four samples of artificial sea water ranging in ionic strength from about 0.07 to 1.0, were titrated. The chlorinity range was from about 2.0 to 29 grams of halides per liter of sea water. The pH of the sea water varied between 7.8 and 8.2. During titrations the solution temperature was maintained constant within 1° between 22° and 25° C.

CALCULATIONS. The pH was calculated using Equation 1, the "glass electrode hydrogen-ion activity", $C_{H^+}f_{H^+}$, according to Equation 2

$$\mathrm{pH} = -\log C_{H^+}f_{H^+} \qquad (2)$$

and the normality of the excess strong acid according to Equation 3

$$C_{H^+} = \frac{(\text{ml. of excess HCl})(N\ \text{HCl})}{(\text{ml. of sample} + \text{ml. of HCl})} \qquad (3)$$

Example. A 100-ml. sample of sea water plus 25.93 ml. of

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Table I. Coefficient Data for Sample, Cl = 18.83 Grams per Liter

HCl, Ml.	pH	$(C_H{}^+ f_H{}^+)(10^5)$	$(C_H{}^+)(10^5)$	$f_H{}^+$
23.93	4.370	4.266	3.31	1.29
24.13	4.280	5.248	4.91	1.07
24.33	4.195	6.397	6.51	0.984
24.53	4.125	7.465	8.12	0.920
24.93	4.015	9.638	11.3	0.854
25.43	3.900	12.62	15.2	0.836
25.93	3.810	15.49	19.2	0.807
26.92	3.670	21.38	26.8	0.798
29.94	3.420	38.11	49.3	0.773
34.91	3.195	63.83	84.4	0.756
39.90	3.050	88.92	117.0	0.760
44.80	2.950	112.5	147.3	0.764

Table II. Summary of $f_H{}^+$ Data

Sample	Cl, Grams per Liter	pH Range	$f_H{}^+$	Average Deviation
1	2.09	4.07–2.93	0.844	0.010
2	2.84	4.00–3.04	0.798	0.006
3	7.03	3.95–2.98	0.764	0.007
4	9.60	4.09–3.03	0.757	0.005
5	12.31	3.62–3.01	0.748	0.007
6	15.38	4.01–2.95	0.746	0.009
7	15.86	3.92–3.03	0.763	0.010
8	16.97	3.69–3.01	0.766	0.016
9	18.83	3.81–2.95	0.777	0.016
10	10.56	3.94–3.08	0.763	0.007
11	23.44	3.98–2.93	0.777	0.011
12	28.93	3.99–3.02	0.768	0.005

0.00999 N hydrochloric acid yielded a solution with a pH of 3.81. The equivalence point occurred at 23.52 ml. of the acid. Therefore,

$$C_H{}^+ = \frac{(25.93 - 23.52)(0.00999)}{(100 + 25.93)} = 19.2 \times 10^{-5} \text{ equivalent per liter} \quad (4)$$

$$C_H{}^+ f_H{}^+ = 15.49 \times 10^{-5} \text{ at pH } 3.81 \quad (5)$$

$$f_H{}^+ = \frac{C_H{}^+ f_H{}^+}{C_H{}^+} = \frac{15.5 \times 10^{-5}}{19.2 \times 10^{-5}} = 0.807 \quad (6)$$

The $f_H{}^+$ values were not constant throughout the pH range investigated; the variations in the values are given for one sample in Table I.

The data for the twelve samples titrated are given in Table II and in Figure 1. The pH range over which the coefficients were sensibly constant is given for each sample.

In these measurements the limiting factor was pH. The reproducibility of the pH measurements was ±0.005 pH unit, which amounts to a 1.2% variation in the $f_H{}^+$ values.

The variation of $f_H{}^+$ with pH is considered to be due to the decreasing ionization of the carbonic acid as the amount of excess hydrochloric acid increases. It is possible, making certain assumptions, to correct for the ionization of the carbonic acid. When this is done the $f_H{}^+$ values at the higher pH values are lowered and the results are in agreement with those at lower values where the excess hydrogen ion is sufficient to repress the ionization of the carbonic acid. However, this must not be done for the present application of the $f_H{}^+$ results.

In Figure 1 the size of the circle indicates the extent of deviation of the individual measurements. From this figure the values of $f_H{}^+$ in Table III have been taken.

Table III. Interpolated Values of $f_H{}^+$

Chlorinity, grams per liter	2	4	6	8	10
Ionic strength	0.072	0.143	0.215	0.287	0.359
Coefficient	0.845	0.782	0.770	0.760	0.755
Chlorinity, grams per liter	12	14	16	18	20
Ionic strength	0.430	0.502	0.574	0.646	0.717
Coefficient	0.752	0.752	0.754	0.754	0.758

DETERMINATION OF ALKALINITY OF SEA WATER

Exactly 25.00 ml. of 0.01000 N hydrochloric acid are placed a clean dry 135-ml. glass-stoppered bottle that has been ag for several months with hydrochloric acid having a pH of abo 3.5. A 100-ml. sample of sea water is pipetted into the bott the sample is brought to the desired temperature, and the p is measured. This measurement may be made aboard ship, b the treated sample may be safely retained and the pH measur more conveniently ashore. As indicated in Equation 7, t normality of the excess strong acid, C_H, is calculated by dividi $C_H{}^+ f_H{}^+$ obtained from the pH measurement of the solution b the appropriate coefficient, f_H, for the water being analyzed.

$$\frac{C_H{}^+ f_H}{f_H{}^+} = C_H{}^+ \quad (7)$$

By substituting the $C_H{}^+$ value in the following equatio developed by Thompson and Bonnar, the alkalinity may b calculated:

$$\text{Alkalinity} = \left[\left(\frac{1000}{\text{ml. of sample}}\right)(\text{ml. of HCl})(N \text{ HCl})\right] - \left[\left(\frac{1000}{\text{ml. of sample}}\right)(\text{ml. of sample} + \text{ml. of HCl})(C_H{}^+)\right] \quad (8)$$

Since all the above factors are fixed except the alkalinity ar the $C_H{}^+$, Equation 8 becomes

$$\text{Alkalinity} = 2.500 - (1250)\left(\frac{C_H{}^+ f_H{}^+}{f_H{}^+}\right) \quad (9)$$

and it is possible to prepare tables or graphs which will rela pH or the $C_H{}^+ f_H{}^+$ and alkalinity for a certain chlorinity rang where no significant variation in $f_H{}^+$ occurs. Furthe more, it is possible to scale a pH meter so tha it will be a direct-reading instrument for th alkalinity or for the normality of the sea wate

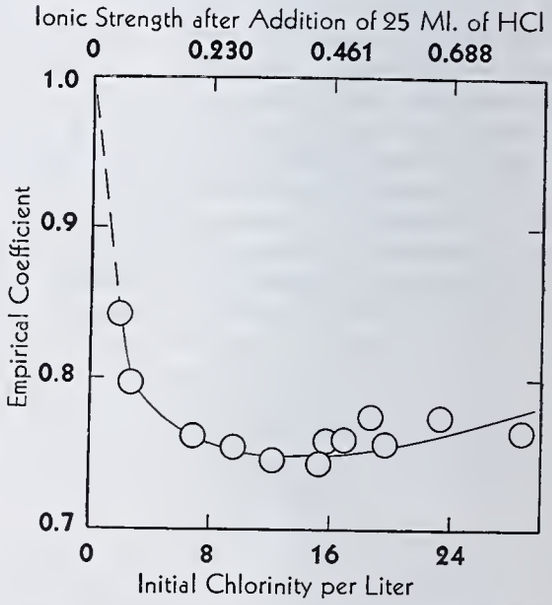

Figure 1. Variation of Coefficient of Hydrogen Ion with Initial Chlorinity of Sample and Ionic Strength after Addition of 25 ml. of Acid

To test the proposed method three samples representing chlorinity of 18.83, 12.31, and 7.03 were treated with severa different amounts of standard hydrochloric acid. The alka linities were calculated and compared with the values obtaine by titration; the results are shown in Table IV.

These results indicate a probable error in the neighborhood o 1%, which is not an unreasonable or unusually high value, con sidering that the concentration of the solution is only abou 0.002 molar with respect to the bicarbonate ion.

The major factors that influence the results of the determi nation have been investigated and the following conclusion

btained. If the pH varies by ±0.01 unit at 3.60 the alkalinity ill vary by ±0.5%. This same percentage variation results om a change of ±3° or a change of ±2.5% in the value of f_{H^+}. n error in measuring the volume of the standard acid of ±0.05 l. and an error of ±2 parts in 1000 in the normality of the tandard acid produce a change of ±0.25%, while an error of ±0.1 ml. in measuring the volume of the sample produces an rror of only ±0.15% in the alkalinity values.

At this laboratory the acid is measured with calibrated autoatic pipets and the sample bottles are usually prepared before he ship is under way. The sample is measured using a measurg pipet described by Thompson and Anderson (*4*). The pH alues usually encountered are around 3.6. Under abnormal onditions they may rise to 4.0 and higher, but this can be emedied by adding more acid, which is a desirable thing to do, s this brings the f_{H^+} values into the region where they are more early constant.

The method described for determining the alkalinity of sea ater routinely should be applicable to a number of other sysms, such as wash waters, effluent from manufacturing operaons, urines, blood, and other biological fluids.

If the procedure is to be applied successfully to other systems, he test solutions must be fairly uniform as to ionic strength nd the concentration of the unknown material. Then by using standard size sample and a fixed amount of reagent, the relaonship between the constituent whose concentration is being etermined and the pH, or any electrode e.m.f. that is related o the test substance, can be determined. Once the relationship as been established, the laborious titration process can be iminated. Obviously, where only an occasional determination to be made, no benefits are found. However, where large umbers of samples are examined in routine industrial or clinical ork, a considerable saving of time is effected.

Table IV. Summary of Calculated Alkalinity Values

Sample	pH of Treated Sample	Alkalinity, Milliequivalents per Liter		Percentage Error
		Calculated	By titration	
A	3.05	2.35		
	3.67	2.33		
	4.13	2.33		
	4.37	2.32		
		Av. 2.33	2.35	1
B	3.01	1.74		
	3.39	1.72		
	3.91	1.70		
		Av. 1.72	1.71	0.5
C	2.98	1.22		
	3.47	1.22		
	3.65	1.21		
		Av. 1.22	1.21	1

ACKNOWLEDGMENT

The authors are especially grateful to T. G. Thompson for his contribution to the preliminary phases of this investigation and to E. C. Lingafelter for his very valuable suggestions and criticisms.

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Accelerated Ozone Weathering Test for Rubber

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ight-energized oxidation and cracking by atmospheric ozone e the agencies chiefly responsible for the deterioration of rubber utdoors. Since these processes are separate and distinct, it is roposed to distinguish between them in the evaluation of rubber for resistance to weathering. An accelerated test for susceptibility to atmospheric ozone cracking is discussed. Apparatus for conducting the test and for measurement of ozone in minute concentration is described in detail.

T HAS been previously shown by the authors (*1*) that two separate and distinct factors are responsible for the changes hich rubber compounds undergo when exposed outdoors: a ght-energized oxidation and attack by ozone which is normally resent in the atmosphere in minute concentration. The former cts independently of stress in the rubber, the latter only when he material is stretched.

Since sunlight, ozone, and temperature vary hourly, daily, asonally, and with locality, the over-all result to the exposed aterial will depend on the balance between the causative factors nd will be unique, since any given combination of such factors ill not be duplicated. It is therefore essential that the susceptiilities of a given compound to damage by these two factors be etermined separately. The information thus furnished will hen permit of an estimate of the durability of the compound nder any given set of conditions.

Damage by light-energized oxidation can be mitigated to ny great extent only by physical protection from light, which an be partially achieved by incorporation of carbon black or rric oxide. It is likely to be the major factor in the case of light-colored goods. Susceptibility can be readily gaged by measurement of the oxygen absorbed when the material is irradiated in air or oxygen under controlled conditions. The present obstacle to formulation of a standard test is the lack of a constant and reproducible light source simulating sunlight.

This article is principally concerned with the ozone factor. In the case of rubber goods containing appreciable loadings of carbon black, such as insulated cable jackets and hose, by far the most important cause of deterioration is the familiar cracking at stressed locations by the ozone of the atmosphere, popularly and erroneously referred to as "light cracking".

Many workers have attempted to use ozone-cracking in the laboratory as a measure of outdoor-cracking, only to abandon the method because of failure to duplicate outdoor ratings. The probable cause of this failure has been found by the authors to be the use of too high concentrations of ozone. Small additions of wax, for example, which would confer substantial protection to cracking at atmospheric concentrations of ozone, are quite without effect in most cases at concentrations one hundred times greater. If ozone concentrations approximating that of the atmos-

Table I. Effect of Temperature on Time Taken to Crack

Compound	Wax	Temperature, Degrees Fahrenheit					
		80	110	120	130	140	150
	%	*Hours*	*Hours*	*Hours*	*Hours*	*Hours*	*Hours*
GR-S cable jacket	0	10	3	1	1	1	1
	1	72	5	2	1	1	1
	2	400	30	6	2	1	1
	3	[a]	[a]	18	5	2	1
	4	[a]	[a]	60	20	3	2
	5	[a]	[a]	240	60	8	3
Natural rubber cable jacket	0	3	1.5	1	1	..	..
	1	100	2	1	1	..	..
	2	300	4	1	1	..	..
	3	450	40	1	1	..	..
	4	[b]	60	1	1	..	..
	5	[b]	85	2	1	..	..
Natural rubber gum compound	0	4	2	1	..	..	..
	1	110	3	1	..	..	..
	2	200	3	1	..	..	..
	3	340	4	1	..	..	..
	4	[c]	5	1	..	..	..

[a] Good after 6 months.
[b] Good after 3 months.
[c] Good after 1 month.

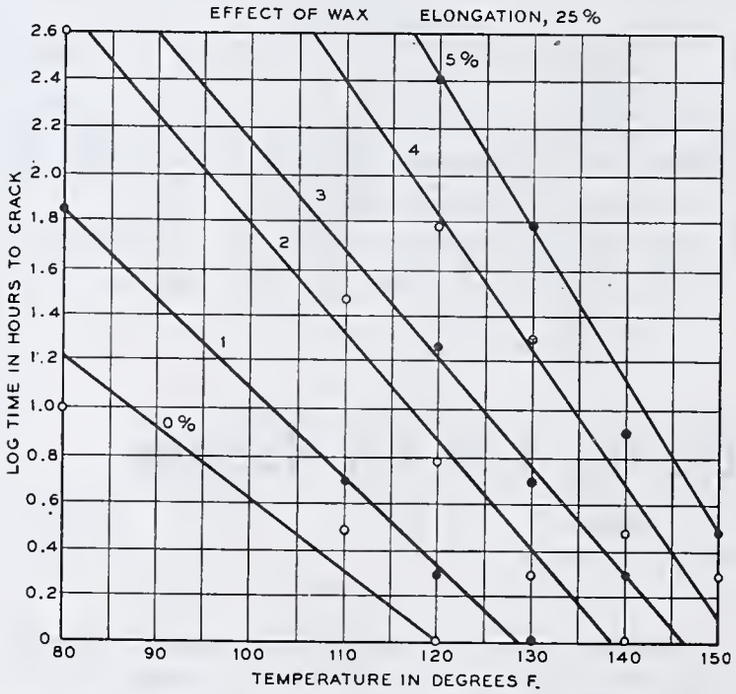


Figure 1. Effect of Temperature on Time Taken to Crack a GR-S Cable Jacket Compound Containing Different Amounts of Protective Wax

Ozone concentration 25 parts per 10^8, elongation 25%

phere be used (around 3 parts per 100,000,000 by volume) outdoor exposures can be closely duplicated. Rubber compounds designed principally as wire and cable insulations for outdoor use have been rated in this way in these laboratories for some time with considerable success.

The nature and degree of cracking of a given compound on exposure to dilute ozone depend on the concentration, the temperature, the degree of elongation, and whether this is static or dynamic (*1*).

TEST CONDITIONS

For accelerated aging in the laboratory the authors have currently adopted a concentration of 25 ± 2 parts of ozone per 100,000,000 of air by volume as the test atmosphere and the expiration of time for the first appearance of cracks in the stressed sample at the chosen degrees of elongation (usually 20 and 30%) and temperature as the measure of performance. The specimen is examined under magnification of 7 to 10 times, which not only gives results more quickly but greatly reduces errors due to any visual defects of the observer.

Ozone concentrations up to 100 parts per 100,000,000 can be satisfactorily used in many cases of highly weather-resistant compounds but are not permissible for general use. Unfortunately in ozone at 25 parts per 10^8, the time taken to crack such compounds at room temperature is too long to be practicable for an accelerated test; so resort is taken to the effect of increased temperature.

In the absence of protective wax, increase in temperature results in more but much finer cracks which in many cases can be recognized only under magnification. In the presence of wax cracks when formed are few but large and well defined. Raising the temperature reduces progressively the effects of the waxes in current usage till a point is reached at which they are ineffectual. For a given wax this temperature increases with its concentration and is higher for GR-S compounds then for natural rubber compounds. For example, a GR-S cable jacket, a natural rubber cable jacket, and a natural rubber gum compound containing various additions of a certain wax, at 25% elongation in an ozone atmosphere of 25 parts per 100,000,000 gave the results shown in Table I in hours to crack.

These values, plotted in Figures 1, 2, and 3 on a semilog basis reveal a distinct connection between temperature and wax content. As the temperature rises a progressive decrease in cracking time occurs, the gap between low and high wax additions being gradually closed, more rapidly in the case of natural rubber compounds than in GR-S compounds. It is this feature that is suggested as the basis of accelerated testing of susceptibility to atmospheric ozone cracking. The temperature of test chosen for a given type of compound is such that the highest economically practicable addition of protective wax can be made to fail in a reasonable time, say 3 or 4 days. This temperature is obviously lower for natural rubber than for GR-S, 70° to 80° and 110° F. being suggested for the former and 120° or 125° for the latter. The proposed concentration of ozone is 25 to 30 parts per 100,000,000 and the degree of elongation 20 or 25%. The criterion of

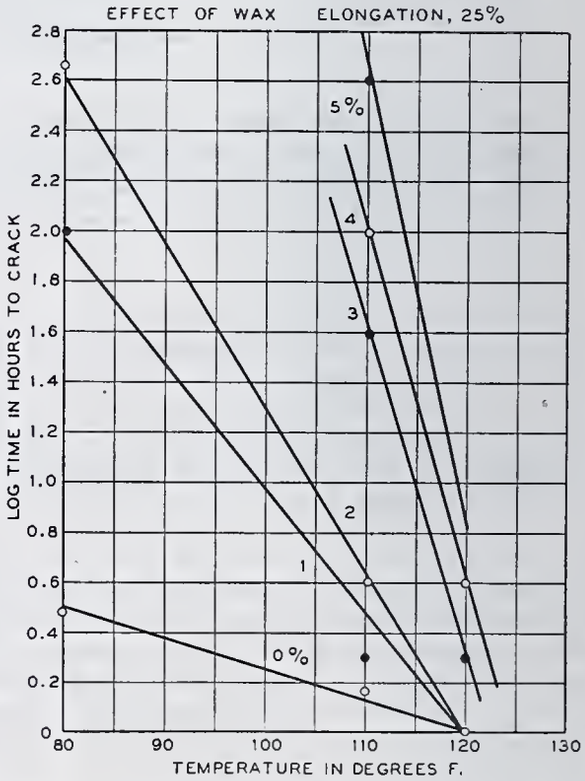


Figure 2. Effect of Temperature on Time Taken to Crack a Natural Rubber Cable Jacket Compound Containing Different Amounts of Protective Wax

Ozone concentration 25 parts per 10^8, elongation 25%

performance is the time taken for the first sign of cracking to appear.

It is not suggested that the ratio of performance between low and high wax additions remains the same with change in temperature, so that the lower temperature life may be determined by extrapolation from the higher. Compounds of known weathering performance must be used as controls. It is considered that as data accumulate over a wider range of compounds it will be possible to establish definite performance requirements under specified conditions similar to those outlined above. The room temperature test is intended to cover compounds of low weathering resistance and to detect anomalies, as, for example, a certain wax examined by the authors which is actually highly efficient at 120° F. but performs poorly at room temperature (Figure 4).

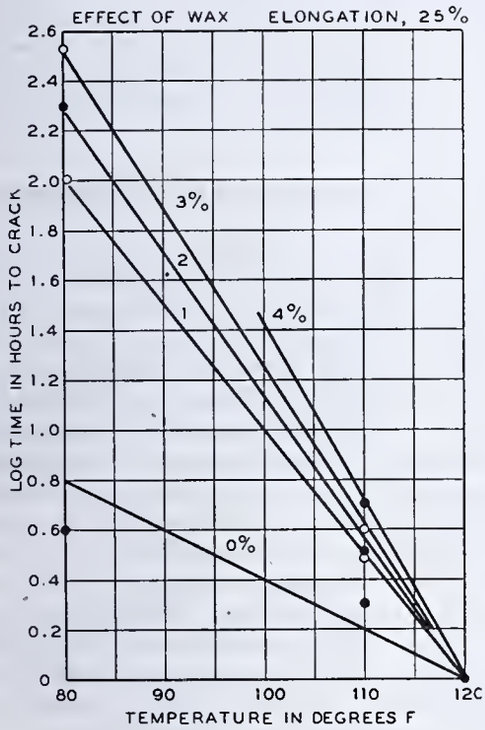


Figure 3. Effect of Temperature on Time Taken to Crack a Natural Rubber Gum Compound Containing Different Amounts of Protective Wax

Ozone concentration 25 parts per 10^8, elongation 25%

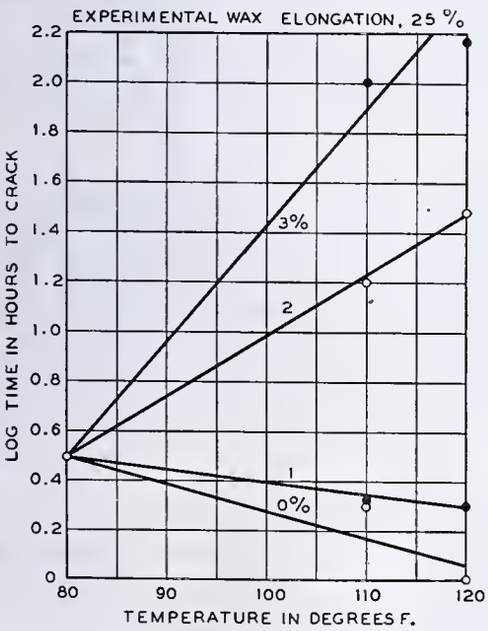


Figure 4. Anomalous Behavior of an Experimental Wax–Natural Rubber Gum Compound

Ozone 25 parts per 10^8, elongation 25%

TEST APPARATUS

Essential to the test are means of furnishing and maintaining uniformly an atmosphere of ozone of the required concentration. This is readily and most conveniently accomplished by passing air or oxygen over a mercury vapor lamp having an envelope of quartz or glass transmitting short-wave ultraviolet light. Ozone is formed from the oxygen by the short-wave ultraviolet light.

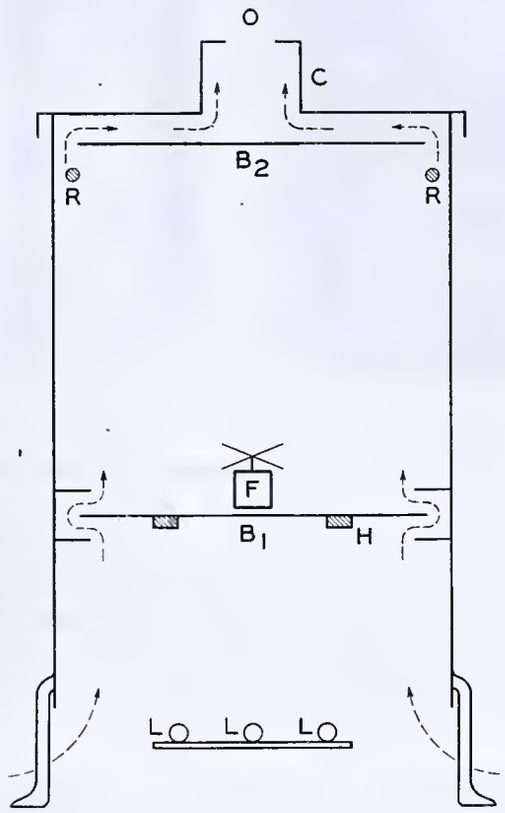
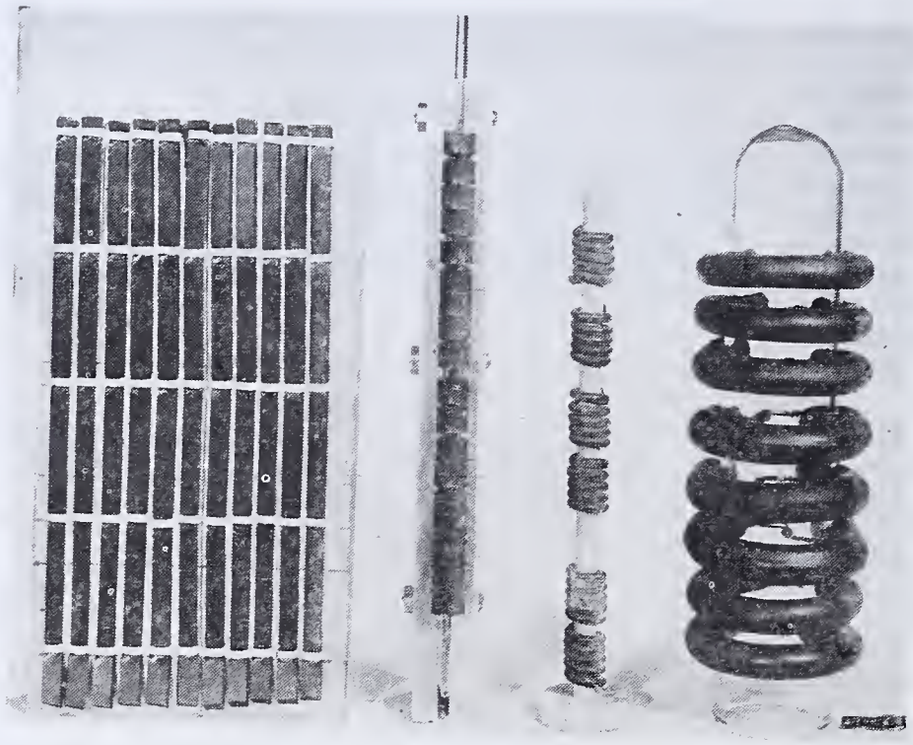


Figure 5. Schematic Drawing of Test Chamber

For laboratory use modifications of the apparatus shown in Figure 16 of the previous paper (*1*) are used. With this arrangement a flow ranging up to 1 cubic meter of air per hour can be supplied at concentrations from 5 to several hundred parts of ozone per 10^8. The higher the flow the lower the concentration. The ozone produced will decrease rather rapidly at first but more slowly after a hundred hours or so, permitting a very uniform output, providing temperature and voltage are kept uniform and the humidity of the air supply has no extreme variations. The lamps should burn continuously. Used in this way they function for several months.

In the past the authors have made considerable use of the G.E. germicidal lamp as ozone generator, since the yield was much less and easier to control than with the quartz tube. However, the nature of the glass in this lamp has recently been modified so as to reduce the ozone output, making it practically useless as an ozone generator. The lamp in current use for the purpose is the Hanovia Safe-t-aire lamp, a mercury vapor discharge tube in quartz. This lamp (Catalog No. 2851) has normally a 30-cm. (12-inch) column and even when operated at as low a voltage as possible generates far too much ozone for easy control. The output can, however, be regulated nicely by covering part of the column with aluminum foil or other opaquing means. Only about 5 cm. (2 inches) of exposed column are needed for a laboratory generator. The tube is held concentric by waxed or shellacked corks or rubber stoppers in a glass tube 2 or 3 inches in diameter provided with side tubes for inlet and outlet of air. This lamp operates at a low temperature and has a very long life. [Currently (August, 1946) a small 12-volt lamp recently developed by the Westinghouse Electric Corp. (Sterilamp WL-794) is being tested. This lamp, of automobile headlight bulb size and intended for use in household refrigerators, is a most convenient source for a laboratory generator delivering from 0.5 to 1.0 cubic meter per hour. Enough experience has not been gained to determine its useful life.]

The flow of ozonized air from these generators is conducted to the base of a laboratory-type oven maintained at the tempera-



a b c d

Figure 6. Methods of Mounting Stretched Specimens for Test

ture at which the specimens of rubber are to be exposed. Suitable baffles should be installed to ensure uniform flow at all parts of the oven, and the inside should be well varnished or waxed, since rapid decomposition of ozone occurs at metal surfaces. Even so, the concentration in the oven will be less than that from the generating apparatus and should always be measured at the location of the specimens.

Where large numbers of specimens are to be exposed a different arrangement is used, depicted schematically in Figure 5.

This consists of a drum or tank, 2 feet in diameter and 3 feet high on legs, open at the bottom and closed at the top, with a loosely fitting lid furnished with a short chimney. The inside is divided into 2 compartments by baffle B_1 12 inches from the bottom. This baffle is 2 inches less in diameter than the drum, the two rings shown acting as a light-lock to prevent light from reaching the upper chamber. Another similar baffle is mounted just below the lid. The lower chamber houses an aluminum plate on which is mounted a single Hanovia No. 2851 Safe-t-aire lamp operated, after aging for a few hundred hours at approximately 750 volts alternating current. Ozone in suitable concentration is thus generated in this chamber. This air rises into the upper chamber through the opening between the edges of baffle B_1 and the sides of the drum, escaping to the outer air around baffle B_2 and through the chimney, C. The amount and concentration of air thus circulating can be readily controlled by adjusting the size of the opening, O, of the chimney or installing a damper in the chimney. A small fan rotating slowly on reduced voltage may advantageously be mounted on baffle B_1 to ensure uniformity of composition and temperature of the air in the upper chamber. The fan blades should be adjusted to generate only a horizontal disturbance.

If the device is to be operated at an elevated temperature, a ring heater is mounted on the under side of baffle B_1, regulated by a thermostat mounted in the upper chamber. A 500-watt heater will maintain a temperature of 110° F. and a 750-watt heater a temperature of 120° F. When used at these elevated temperatures the drum is insulated with a layer of thick felt or other suitable material. The specimens to be exposed are suspended by wires from rail R around the inside walls of the upper chamber. The drum should preferably be of aluminum, which is less active as a decomposition catalyst of ozone than copper, iron, or zinc. In any event the inside should be painted with a thin layer of wax. In the units used in these laboratories a full chimney opening gives a concentration of ozone in the upper chamber of 25 parts per 10^8, using the lamp referred to. The opening is reduced in size as the activity of the lamp decreases with age.

Specimens for exposure are mounted shown in the photograph of Figure 6.

Sheets are mounted as strips stapled to waxed board, a, at elongations chosen, usual 20, 25, 30, and 50%, though GR-S compoun usually break at the staple with 50% elong tion. Alternatively strips are mounted be around a mandrel of a diameter calculate to give the required elongation, the en secured between 2 wooden strips, b. Wires, are wound around waxed dowels or glass ro of the necessary diameter and cables, d, sim larly wound on glass tubing. Specimens mu not be exposed immediately after mountin but must stand for a uniform time of at lea 24 hours to allow healing of any wax film th may have been disrupted by the stretchin Comparison between sample and control shou be on the basis of identical mountings. Shee should not be compared with wires, for e ample.

MEASUREMENT OF OZONE CONCENTRATION

The above procedure is of little value wit out a means of determining the concentratio of the ozone in the atmospheres in the exp sure chambers. In the routine operation several of the accelerated weathering devic described, a simple and rapid method of est mation had to be devised, because although wit careful attention to air flow, voltage, and temperature, uniform concentrations can be maintained, ozone is an unstable substanc and assurance of a uniform concentration can be had only by re peated checking at reasonably close intervals, at least daily Moreover, the answer must be obtainable within a relatively short time, not more than an hour. The ideal method would b by measurement of ultraviolet light absorption in the region o the ozone absorption band having a peak at 2550 Å. However, a the concentrations of ozone in use this would call for a path a least 50 feet long to give a practicable measure and is thus incon venient.

The method currently used reverts to the classical method o estimation by absorption by a solution of potassium iodide i water and estimation of the iodine liberated. To furnish suffi cient iodine for measurement in the short time allotted a larg volume of air must be dealt with. To ensure absorption of th ozone, the air to be measured is made to generate a fine spray o potassium iodide solution. In this way an enormous surface o solution is furnished for the reaction. The apparatus is show schematically in Figure 7 and illustrated in Figure 8.

In Figure 7, A is a glass tube 0.375 inch in diameter (approxi mate) and about 4 inches long, terminating at B in a short length of capillary tubing with a bore of 1 to 2 mm. Concentric within A is a smaller glass tube, C. (Figure 7, a, shows this assembly on a larger scale.) The end of C is first carefully heated in a blow pipe flame till the bore is reduced in size so as just to admit a No. 69 drill. At this thickened end two flats are ground off on a sheet of fine Aloxite paper as at D in Figure 7, b. When in position in tube A, end D fits snugly against the hole in capillary B. C may now be sealed to A at the upper end, but it is better to rely on the rubber connection at E to hold the tubes in place, since once sealed in, C cannot be removed for cleaning in the event of a blockage.

F is a trap about 2 inches in diameter and 4 inches long, requiring no further description, and G is an enlargement in the exit tube, about 1.5 inches in diameter, containing glass wool to trap spray passing F. F is connected to the side tube of A by rubber tubing or may be permanently attached, as shown in the figure. The rubber connector is more convenient, but the tubing used must first be soaked for a long period in dilute iodine solution and thoroughly washed, or iodine may be taken up from the reagent. H is a 1-liter three-necked Woulff's bottle in which A and F are secured by Pyrex ground joints, A occupying the center opening with B protruding just below the neck and tube J

reaching to within 0.5 inch of the bottom of the bottle. The third neck serves to introduce and remove the reagent.

A is connected through rubber joints and glass or plastic tube *K* to rotameter *L*, graduated from 0 to 1.0 cubic meter of air per hour. The entrance to the rotameter is connected to the atmosphere whose ozone content is to be determined and the exit from *F* is connected to a vacuum line. After 75 ml. of reagent are introduced into *H*, the stopper is replaced, and the vacuum gradually applied. Almost the entire body of liquid will enter *F*, furnishing a head of reagent at *B*, where the entering air resolves it into a fine mist which fills the entire bottle. At the end of the run the vacuum is disconnected and the liquid transferred to the titration vessel. For atmospheres containing around 25 parts of ozone per 10^8 of air, ample iodine for titration will be obtained in 0.5 to 1 hour. When runs longer than 1 hour are called for, it is necessary to add distilled water at intervals to make up for evaporation. This is most conveniently done through the air intake. The liberated iodine is determined by titration with sodium thiosulfate. Since the amount is so small, 0.002 *N* to 0.001 *N* solutions must be used, and since the end point using starch as indicator is uncertain, the electrometric method of Foulk and Bowden (*3*) is resorted to, in which use is made of the depolarizing effect of iodine on a polarized electrode.

In the authors' practice the titration vessel is a 250-cc. wide-mouthed extraction flask having a hole in the side near the neck. A two-hole rubber stopper carries into the flask 2 glass tubes into which are sealed the two electrodes, in this case stout platinum wires (0.1 inch thick) with circular loops at the ends to increase the areas exposed to the liquid. Sensitivity is increased by increasing the surface area of the electrode, but this form is used because it is rugged and not disturbed by agitation of the liquid. To the electrodes is applied a potential of 30 to 40 millivolts. This is readily obtained by connecting suitable resistors—e.g., 30,000 and 1000 ohms—in series across an ordinary 1.5-volt dry cell and picking off the voltage across the resistor of lower value. A galvanometer is connected in series. The authors use a Rubicon 3402-H.H. with an Ayrton shunt, but a less sensitive type is probably sufficient.

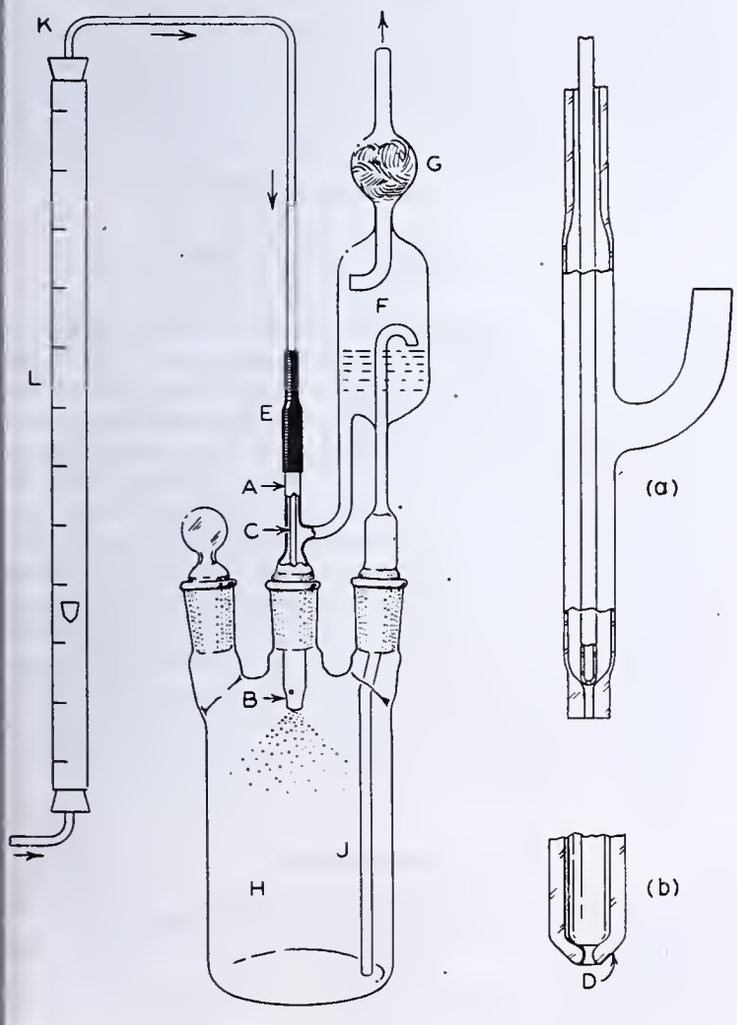


Figure 7. Ozone-Absorbing Device

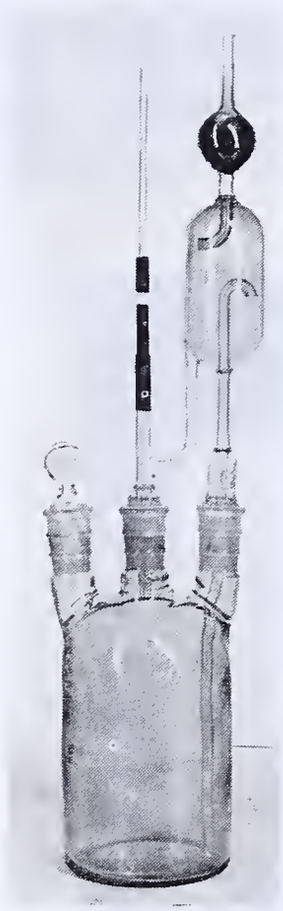


Figure 8. Ozone-Absorbing Device

Fifteen grams of potassium iodide are dissolved in 75 ml. of buffer solution (equal volumes of 0.025 *N* disodium hydrogen phosphate and 0.025 *N* potassium dihydrogen phosphate). The solution is introduced into the titration flasks, the electrodes are inserted, and the liquid is swirled vigorously over them. Following an initial kick the galvanometer spot will return to zero if no iodine is present, because polarization of the electrodes will prevent passage of current. Presence of an oxidizing agent such as iodine removes the polarizing hydrogen from the cathode and current flows. Addition of thiosulfate (through the hole in the side of the flask) till the iodine is removed restores the polarized state and returns the galvanometer deflection to zero. The reagent will usually require the addition of 2 to 5 drops of 0.002 *N* thiosulfate, depending on the batch, to bring this about.

After the ozone run the iodide solution containing the iodine is placed in the titration vessel and thiosulfate is added until only a barely perceptible yellow remains; then the electrodes are inserted and thiosulfate is added drop by drop at intervals till no deflection is obtained, the liquid being vigorously swirled meanwhile. The liquid is then returned through the trap to rinse the apparatus and the titration is completed. One cc. of 0.001 *N* thiosulfate represents 0.0112 cc. of ozone at S.T.P.

A little difficulty may be encountered at first in identifying the end point to within one drop of thiosulfate solution at this low concentration. It will be found easier if the titration is made to a small residual deflection of the galvanometer.

Neither the form nor dimensions of the apparatus described are critical. Those given are of the apparatus in current use. Duplicate apparatus reproduces results within ±5%, which is good enough at these low concentrations. It has been found that reducing the concentration of the potassium iodide solution below 20% gives low results. The system described passes about 0.3 cubic meter of air per hour, but this can be changed by changing the size of the air jet, *C*. With a given jet there may be considerable leeway in the size of the capillary nozzle. The criterion is a reaction vessel filled with a mist of reagent. The authors have compared reaction vessels ranging in size from 125 ml. to 12 liters and find a tendency to low results with a capacity of less than 500 ml. A larger vessel than this yields no advantage.

A most important point to remember is that potassium iodide in solution is photochemically oxidized to iodine in presence of light, even in neutral or alkaline solution. Therefore titration must not be conducted in bright daylight and during the ozone run the whole of the absorption apparatus must be enclosed in a light-tight box as shown in Figure 9. Failure to observe this precaution will result in utterly erroneous findings.

The 0.002 *N* thiosulfate should be standardized at frequent intervals, as such dilute solutions lose strength through oxidation.

Since the iodine in solution has a vapor pressure, some will be carried away in the exhausted air; a correction must therefore be applied to the result obtained. The amount of this has been determined in two ways: (1) by making determinations over a range of increasing periods of time, plotting the ozone found against time, and extrapolating to zero time which gives the true value; (2) by operating the apparatus with a stream of nitrogen

and simulating the ozone reaction by adding small amounts of 0.001 *N* iodine at frequent intervals over the desired period and in amount equivalent to the ozone concentration studied. Comparison of the iodine remaining in solution with the amount added gives the error. These two methods checked each other within ±5% at any concentration between 3 and 25 parts of ozone per

Figure 9. Dark Housing for Ozone Absorber

10^8. The percentage loss with time is shown by Figure 10. The determinations were made over a period of only 2 hours at the highest concentration, since measurement time in practice never exceeds 1 hour. With decreasing concentration the period was increased progressively up to 8 hours for the lowest.

The curve applies only to the particular conditions employed—viz., a flow of 0.27 to 0.30 cubic meter per hour and a vacuum of 60 cm. of mercury. For other conditions the correction must be determined experimentally.

The absorption of ozone is assumed to be complete, since increasing the concentration of potassium iodide up to 80% gave no higher values. Further, dilution experiments, in which ozone of concentrations up to 100 parts per million was diluted with a known volume of air and the dilution simultaneously measured, when carefully conducted, checked within ±5%. This is not a definite proof but a reasonable assumption.

It might be thought that the loss of iodine could be reduced by cooling the reaction vessel in ice. The reduction has proved on trial to be insignificant. Expansion of the air at the nozzle and some evaporation of the water results in cooling the reagent to around 60° F., depending on the duration of the run. Where room temperatures are high as in summer months, however, immersion in ice would ensure more uniform conditions.

The principal merit of this method lies in the rapidity with which ozone concentrations at the level suggested for use in the accelerated weathering methods described can be made. Since variations in the rubber compounds tested and the personal errors

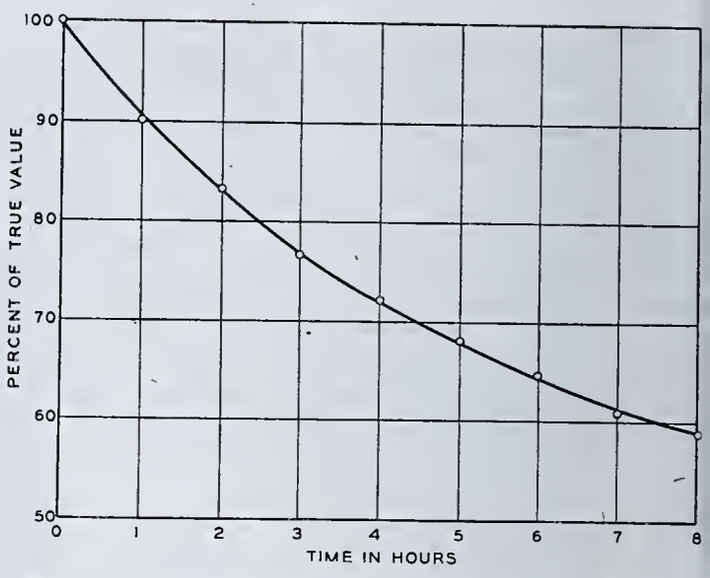


Figure 10. Correction Curve for Ozone Estimation Described in Text

in recognizing the first appearance of cracking are often large, i is felt that this method is well within the over-all accuracy pos sible in weathering values. It is planned to check the metho further against light-absorption determinations.

An apparatus for the continuous recording of ozone concen tration, employing the above principles, has been described by Gluckauf *et al.* (*4*). The output of such an apparatus coul readily be arranged to maintain a constant concentration of ozon in a test chamber by adjustment of the voltage to the ultraviole lamp. It is possible, however, that the effort involved in main tenance of such apparatus would exceed that called for by inter mittent control in the procedures described in this paper.

In the measurement of ozone concentrations of the atmosphere by the potassium iodide reaction, the criticism is often advanced that other oxidizing agents known to be present also liberate io dine from potassium iodine. These are nitrogen oxides, hydro gen and other peroxides, and chlorine. However, Gluckauf *et al.* (*4*) point out that the reaction producing iodine

$$NO_2 + 2H^+ + 2I^- \longrightarrow H_2O + NO + I_2$$

proceeds only to a very limited extent at low concentrations and at pH 7.0, because of the lack of hydrogen ions and prove this by experiment. The present authors have found by trial that nitrogen peroxide even in concentration three- or fourfold that of the atmosphere (*2*, *5*) does not liberate iodine from potassium iodide by the procedure described. As for hydrogen and other peroxides, the authors have made repeated attempts to trap suspended particles of organic peroxides in a water spray and to freeze out hydrogen peroxide in traps cooled by a dry ice–acetone bath. The liquids so obtained from several cubic meters of air have invariably failed to liberate titratable amounts of iodine from buffered potassium iodide solution. Free chlorine apparently is absent from the atmosphere at the location of these laboratories. Nevertheless, in this connection the possibility of the presence of these or other oxidizing agents in the atmosphere of industrial locations should be borne in mind.

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Determination of the Relative Acidity of Wood and Adhesive Joints

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Problems involving the assessment of the pH of wood glue and plywood sections are discussed. The use of fluorescent dyes and ordinary color indicators has been investigated and very interesting observations are described. Dye indicators are shown to be satisfactory for differentiative pH measurement.

ONE of the important considerations in investigating the character of the glue line in plywood bonding is the acidity of the actual glue and the adherent wood. It is known that corrosion of the wood takes place when the glue used is either too acid or too alkaline (*1*, *3*, *5*), but, as yet, research workers on the subject have found no suitable solution of the problem of assessing the actual acidity at the glue line after pressing and curing the plywood. This paper discusses some of the problems involved and proposes a method which, for all practical purposes, gives the pH of the finished plywood glue line.

The "reference pH" used by Campbell (*4*), grinding up the plywood and suspending it in water or acid, is not suitable for attacking most problems concerning glue lines because too many factors affect its determination. For example, the thickness of the veneer, the quantity of the resin used, the degree of dilution, the leaching out of salts from the wood, and the possible hydrolysis on dilution would all have an influence on the final pH measured with the glass electrode. At best, the reference pH gives an over-all average of the acidity of the veneers and the glue together, but affords no specific information as to the actual acidity at the wood-glue interface. Campbell said that the pH of the moisture condition of air-dry wood could not be measured directly because wood in this condition is not moist enough to wet the glass electrode. He overlooked, however, the possibility of using suitable indicators to determine acidity by the colorimetric method.

Table I. Fluorescent Dyes

	Dye	Manufacturer	Color Change
1.	Coumarin	Dr. Theod Schuchardt-Gorlitz C.P.	None
2.	Fluorescein sodium	B.D.H.[a]	Very slight
3.	Eosin	Eastman Kodak	None
4.	Erythrosin	Eastman Kodak	None
5.	1 - Naphthol - 4 - sulfonic acid	C.A.F. Kahlbaum, Chemische Fabrik Adlershof bei Berlin	Slight
6.	2 - Naphthol - 6 - sulfonic acid	Eastman Kodak	None
7.	Naphthionic acid	Eastman Kodak	None
8.	Amino G acid, 2-naphthylamine - 6,8 - disulfonic acid	C.A.F. Kahlbaum	Good
9.	R acid, 2-naphthylamine-3,6-disulfonic acid	C.A.F. Kahlbaum	Good
10.	1 - Naphthylamine - 4,8-disulfonic acid	C.A.F. Kahlbaum	Good
11.	2-Naphthol	Du Pont	None
12.	2-Naphthol	C.A.F. Kahlbaum	None
13.	1-Naphthylamine	Du Pont	None
14.	Acridine	B.D.H.	Slight
15.	Auramine	Du Pont	Slight
16.	Potassium thiocyanate	B.D.H.	None
17.	Uranyl nitrate	Schering Kahlbaum	None
18.	Phenol red	B.D.H.	None
19.	Rhodamine B	B.D.H.	None
20.	Rhodamine 6DGN extra	Du Pont	None
21.	Basic orange 3 RN	Du Pont	None
22.	Dihydrothio-*p*-toluidine	Du Pont	None
23.	Berberine sulfate	B.D.H.	Slight
24.	Ammonium salt of phenyl per acid	Du Pont	None
25.	Erythrosin bluish	National Aniline & Chemical Co.	Good
26.	Eosin bluish (alcohol solution)	National Aniline & Chemical Co.	Good
27.	Auramine	B.D.H.	None
28.	Rhodamine 6G	B.D.H.	None
29.	Tetrahydroxyflavonal	B.D.H.	None

[a] British Drug Houses.

FLUORESCENT INDICATORS

Some of the more common titration indicators gave very poor and indistinct color changes when applied to wood and therefore the use of ordinary color indicators was at first discarded. D. H. Hamly of the Department of Botany suggested the use of fluorescent dyes, and was of invaluable assistance in the subsequent work in fluorescent microscopy.

The working principle was that the fluorescent color of the dyes under ultraviolet rays would not be affected by the color of the wood. Little information could be found in the literature regarding fluorescent dyes which manifested a change in color with a change in pH. Consequently, the work began with a search for fluorescent dyes that might be suitable as indicators.

All known available fluorescent dyes (see Table I) were investigated.

A solution of each dye was made up, 1 to 1000 where possible and more dilute when necessary, and kept in a stock bottle. Strips of birch veneer 3 × 0.5 × 0.0625 inch were used as test pieces and their pH was controlled by soaking in solutions of different acidities. Standard buffers, hydrochloric acid, sulfuric acid, and sodium hydroxide, were employed in making the standard pH solution. After soaking for 24 hours, the test pieces were allowed to dry in air. A drop of the dye was put on the surface of each of several test pieces of varying pH, and when the dye was dry, the resultant color was observed under the fluorescent microscope.

Five dyes (Table I) showed some appreciable change in color with varying pH, but in each case the color change was not sharp, but gradual; furthermore, there was actually only a change in shade of color rather than a distinct change from one color to another. Attempts to set up standards in order to determine the actual range of the color variation failed because there was no method of determining the true pH of the birch test pieces. The use of a material other than wood as a background for the dyes was found to be impractical because the character of the background has a marked effect on the fluorescence. In view of these facts, it was decided to abandon the investigation on the use of fluorescent dyes for the examination of the glue line. Research was continued, however, with the ordinary color indicators and some very interesting results were obtained.

COLOR INDICATORS

It was first noticed that B.D.H. (British Drug Houses) Universal indicator, which is green in its ordinary state, changed to red immediately when it came in contact with filter paper. The dye was applied to a strip of birch, and again a red color indicated acid constituents. The acidity cannot be due to the carbon dioxide in the air, because Universal indicator does not turn red when left for a long period of time in distilled water exposed to the atmosphere. The actual pH of the birch could not be determined with this indicator because it is not suitable for pH's lower than 4. A search was begun for an indicator with the proper pH range, which would also exhibit a good color change on wood.

BROMOPHENOL BLUE AND THE pH OF BIRCH. Investigation of all the dyes with a color change below pH 4 showed that bromophenol blue was the only one suitable for measuring the

acidity of birch. This dye has a range from 3.0 (yellow) to 4.6 (blue) with a gradation of the combination of these two colors between the limits.

A series of buffers was made which covered the pH range from 3.0 to 4.6 in steps of 0.1 unit. Strips of birch veneer were cut and soaked in these buffers for 1.5 hours. This period was long enough to condition the test pieces, since longer soaking had no influence on the final pH of the strip. It will be assumed that the pH of these strips attains the same value as the buffer, because the color indicated on the wood with bromophenol blue compared very favorably with the color in the buffer solution, and since the amount of buffer used was great in comparison with the size of the test piece, the pH of the wood must eventually, by leaching and impregnation, reach the pH of the buffer. The wood constituents are not sufficient to affect the pH of the buffer.

The soaked strips, when dry, were painted with bromophenol blue and served as standards for the comparison of color changes of the dye on wood. The pH of birch was then determined by painting a clean birch strip with the indicator and comparing the resulting color with the standards.

Because the dye solution was thought to have an effect on the pH of the wood, experiments were carried out using various concentrations of dye and varying alcohol-water mixtures as the solvents. It was found that a concentration of 1.2 grams of bromophenol blue per liter was sufficient to give a good color on wood. The optimum solvent proved to be 70% alcohol by volume, since this gives a dye solution with a pH very close to that of the wood itself. With this solvent the indicator reaches a permanent color in a minimum time. Other solvents with more or less water percentages give the same results, but longer time is required to obtain a reading.

The wood test pieces should be clean or preferably recently planed, because handling of the wood has a ready and pronounced effect on the pH at the surface. Another interesting observation was that a test strip, even when clean, shows a slight variance in pH over different areas. This led to the investigation of moisture content of the wood.

MOISTURE CONTENT OF WOOD

In sapwood, the moisture is present in three conditions: (1) as the chief constituent of the sap, (2) absorbed in the cell walls, and (3) filling, completely or partially, the intercellular cavities (*2*). In heartwood, however, it occurs only as condition 3. It is obvious, then, that the moisture content of wood varies over different areas. If moisture content has an effect on the pH of the wood, it should not be surprising that bromophenol blue shows varying pH in a sample of wood. Experiments were carried out to find the effect of moisture content on the pH of wood.

A newly planed test piece was painted with the indicator and then cut into three equal sections. One section was put in a closed box saturated with water vapor; the center section was allowed to stand in the atmosphere; and the third section was put in an oven at 70° C. After 24 hours, the colors of the three sections were compared. As was expected, the pH of the heated section dropped, and the pH of the humidified section rose, while the center section remained the same and served to show that there was a definite change in each of the other two.

Quantitative values of the pH could not be determined because the color limits of the indicator were approached. This proved, however, that oven-dry wood and the "saturated" wood had a difference of at least 1.5 pH units in the case of yellow birch. In a matter of a few hours, the surface of the wood regained its original air-dry moisture content.

The establishment of a definite relation between moisture content and pH of wood is not practical, in view of the fact that the moisture content varies in different woods and in different parts of the same wood. The fact remains, however, that the value of the pH is very sensitive to moisture content. Even the changing humidity conditions of the atmosphere from day to day produce a corresponding change in the pH of the wood. This indicates that the buffering system in wood mentioned by Campbell and Packman (*4*) cannot be very effective in regard to humidity changes.

pH IN DIFFERENT WOODS

Samples of several different woods were obtained from the Department of Forestry and tested with bromophenol blue. The intrinsic dark colors of some species made it difficult, but, for the most part, readings were possible. Values ranged from 3.2 (B.C. cedar) to 4.5 (white ash) (see Table II). Values are by no means absolute, because pH may vary in each species and readings were made using the buffered birch samples as standards.

No relation could be found between pH and the listed moisture content of various species, nor was there any relation between pH and specific gravity. There was no appreciable difference in the average pH of the two types—i.e., coniferous and deciduous.

pH OF THE GLUE

The pH of the glue before application can usually be measured with the glass electrode. Data relating to the effect of the pH of the glue on the strength of plywood are tabulated by Dowling (*5*), but his measurements were made before pressing and he did not attempt to measure the ultimate pH of the glue after curing and setting.

Experiments were carried out with color indicators in an attempt to add the dye in the glue mixture and determine the pH after setting. With dark-colored glues like phenol-formaldehyde the method was impractical, but with cold-setting glues like urea formaldehyde favorable results were obtained. It was observed that, because of reaction of the catalyst, the pH dropped as polymerization progressed in the case of urea-formaldehyde. No change was noticeable in the pH of casein on setting.

pH AT THE GLUE LINE

Experiments were then carried out involving the application of color indicators to the glue and wood in cross sections of plywood.

Table II. Relative pH of Different Wood Species

Species	Specific Gravity	Moisture Content, %	pH
Coniferous			
Balsam (eastern)	....	....	4.0
Cedar (B.C.)	....	....	3.2
Cedar (eastern)	....	....	3.5
Cypress	....	....	3.9
Fir (B.C.)	0.53	4.55	3.4
Hemlock (eastern)	....	....	3.8
Hemlock (western)	0.45	....	3.6
Larch (white)	....	....	3.6
Pine (eastern white)	0.42	....	3.4
Pine (jack)	0.51	....	3.7
Pine (red)	....	7.3	3.7
Redwood	....	....	3.6
Spruce (black)	0.43	....	3.4
Spruce (Sitka)	0.54	....	3.5
Spruce (white)	0.42	....	3.9
Tamarack (eastern)	0.59	....	3.6
Deciduous			
Ash (black)	....	....	3.7
Ash (white)	0.66	....	4.5
Basswood	....	....	3.4
Beech	....	....	3.3
Birch	0.64	5.7	3.8
Cherry	....	....	3.5
Chestnut	....	....	3.2
Elm (white)	0.59	....	3.6
Hickory	0.80	....	3.2
Maple (hard)	0.78	....	3.6
Maple (soft)	....	....	3.8
Oak (red)	....	....	3.3
Oak (white)	0.72	6.2	3.5
Poplar	0.43	5.8	3.9
Sycamore	0.56	....	4.1
Walnut	....	5.4	3.5
Whitewood	....	....	3.9
Willow	0.40	....	4.4

Chlorophenol red was painted on the cross section of a finished sample of three-ply birch bonded with alkaline phenol formaldehyde. After the dye was dry the specimen was examined under the microscope. Beautiful distinction was manifested between the glue line and the veneers. The glue line itself and the wood at the interface turned a bright red color (indicating a pH of approximately 9), while the wood not in contact with the glue was yellow. Penetration of the glue into the veneer became apparent and single tendrils of resin stood out clearly. Thus a good picture of the glue line and veneers in cross section was produced, showing the distribution of the glue and the effect of the alkaline constituents of the glue on the wood in contact with it.

The same indicator was applied to the surface of a veneer in the same sample of plywood. Penetration areas were well defined and in some cases penetration not visible without the indicator was brought out.

With urea-formaldehyde the effect was not so clear, because the pH of the birch itself is too nearly that of the glue line and no clear distinction was visible. Bromophenol blue was found more suitable for urea resins. With casein both chlorophenol red and bromophenol blue are satisfactory.

The application of bromophenol blue to the glue line readily distinguished an alkaline glue. Urea-formaldehyde and casein can thus be differentiated, even though they are similar in appearance. The phenolics are distinguishable by their dark color.

Color photographs were taken of typical specimens and good results were obtained. The authors will be pleased to make the films available to interested readers.

SUMMARY

The use of fluorescent dyes as pH indicators for plywood has been investigated and found to be impractical. It is possible to determine the relative pH of wood by the use of ordinary color indicators.

Experiments have proved that the pH of any wood species is closely related to its moisture content and wood structure. The pH of light-colored glues was measured even after setting.

Color indicators have yielded valuable information regarding pH and glue penetration in plywood.

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Determination of Dipentaerythritol in Mixtures of Pentaerythritol and Dipentaerythritol

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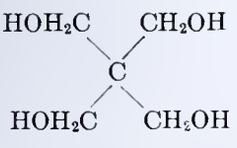
BECAUSE pentaerythritol and dipentaerythritol are now established as raw materials for the resin chemist, greater accuracy in the analysis of commercial pentaerythritols is becoming of prime importance.

Pentaerythritol (also called monopentaerythritol) has the formula

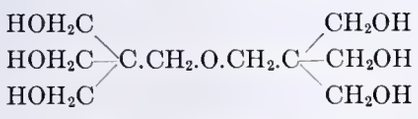


and is usually sold as a "pure" product or in admixture with varying proportions of dipentaerythritol.

The purest form of pentaerythritol usually sold commercially contains about 2 to 3% of dipentaerythritol and about 0.1 to 0.2% of tripentaerythritol. It usually consists of clear, colorless octahedrons melting at about 254+° C. Strictly pure pentaerythritol melts at 260.6° C.

The purest form of dipentaerythritol sold commercially contains about 8 to 10% of tripentaerythritol, a minute amount of tetrapentaerythritol, and possibly a few tenths per cent of monopentaerythritol. It usually exists in the form of colorless, opalescent, platelike crystals of melting point about 218–220° C. Strictly pure dipentaerythritol melts at 221° C.

The chemical formula for dipentaerythritol is:



In addition to pure pentaerythritol (which is also often referred to as nitration grade or as high melting point pentaerythritol) and pure dipentaerythritol, various mixtures of pentaerythritol and dipentaerythritol, such as the 90-10, 85-15, 75-25, and 70-30 mixtures, have found their way into industry. Consequently, there is a real need for a satisfactory method for the determination of dipentaerythritol in such mixtures.

The benzilidine, the various acetyl procedures, and other methods based on the determination of hydroxyl groups have been tested and found wanting in some important respect.

The method described below has been developed and used in the laboratories of the Trojan Powder Company for a number of years, with results which have been more reliable and meaningful than those obtained by any heretofore known procedure. An understanding of certain phenomena related to aqueous solutions of the pentaerythritols will aid the chemist using the method in obtaining reasonably accurate and duplicatable results.

Aqueous solutions of the pentaerythritols are not what the physicist would call ideal solutions, but are very complex mixtures, depending upon their proportions, concentrations, and temperatures. Consequently, such properties as the solubility of each pentaerythritol in water, the solubility of dipentaerythritol in aqueous solutions of monopentaerythritol, the solubility of tripentaerythritol in solutions already containing monopentaerythritol and dipentaerythritol, etc., and the nature of the crystals obtained from these solutions are not expressible in simple terms. There is no doubt that molecular complexes and equilibrium mixtures play an important function in aqueous solutions of pentaerythritols and the phenomenon of hydrogen-bonding seems to be an underlying cause.

The present method for the determination of dipentaerythritol is based upon the precipitation of dipentaerythritol from aqueous solutions containing a mixture of monopentaerythritol and dipentaerythritol, such precipitation being effected by ensuring the presence of at least a certain minimum ratio of dipentaerythritol to pentaerythritol in the clear solution before any precipitation, or crystallization, is allowed to take place. This minimum ratio

of dipentaerythritol to pentaerythritol is provided in many cases by actually adding a weighed amount of pure dipentaerythritol to the sample and then applying a corresponding correction on the final, weighed dipentaerythritol crystals obtained, to allow for this added dipentaerythritol. In terms of units of weight, the minimum proportion of dipentaerythritol to pentaerythritol which has been found operative in this method is about 1 part of dipentaerythritol to 1 part of pentaerythritol—to a 2.5-gram sample consisting of 2.0 grams of pentaerythritol and 0.5 gram of dipentaerythritol, one should add at least 1.5 grams of pure dipentaerythritol before proceeding with the analysis. The procedure given below is recommended as generally applicable over a wide range of commercial pentaerythritol products.

METHOD

Exactly 2.500 grams of the sample (in a tared aluminum dish) and 2.500 grams of pure dipentaerythritol are weighed out. The sample is transferred to a weighed (to the nearest 0.1 gram) 125-cc. Erlenmeyer flask, 60 grams of distilled water are added and the flask is shaken gently, and heated (on an electric hot plate) to take all the sample into solution. To prevent undue loss of water during this heating, a clean rubber stopper should be placed in the neck of the flask and loosened a few times to relieve any excess pressure in the flask. The heating and shaking should be continued 3 to 5 minutes, until a clear solution results.

If an undue amount of water has been driven out of the flask during this dissolving stage, the flask with its contents should be placed upon a balance and water added, drop by drop, to ensure the presence of 60 grams of water. The flask should be allowed to stand in the room for at least 0.5 hour, with occasional gentle shaking, then transferred to a water bath at a temperature of 18° C. (not less than 15° C.), and allowed to remain here overnight.

The next morning the total weight of the flask and its contents is checked to be certain that 60 ± 0.1 grams of water are present in the mixture. Usually the addition of a few drops of water is necessary.

The flask with its contents is now placed in a water bath at 25° C. and gently shaken; the stopper is removed, and the crystals of dipentaerythritol which have separated are stirred by means of a glass rod, keeping the contents at 25° C. When the crystals have been broken up and the mixture has been at 25° C. for at least 10 minutes they are transferred to a weighed 15-ml. Gooch-type, fritted-glass crucible of medium porosity. This transfer should be made by means of 40 ml. (measured) of denatured alcohol (Formula 1) applied by means of a small wash bottle. The crystals are then washed with 10 ml. of ethyl ether and dried at 110° C. for 1 to 2 hours, or to constant weight.

CALCULATIONS

The weight of dipentaerythritol actually in the original sample is calculated from the final dry weight obtained by first deducting the weight of pure dipentaerythritol added and then applying a correction for the amount of dipentaerythritol remaining in the filtrate and alcohol washings. This correction has been carefully determined and found to be 0.145 gram for the conditions specified above. If these conditions are altered, this value will be different.

Method of Calculation.

$$\% \text{ dipentaerythritol} = \frac{A - 2.500 + B}{2.500} \times 100$$

where A = weight of dry crystals obtained and B = solubility correction, or in this case, 0.145 gram

The 2.500 figure in the numerator is the weight, in grams, of the added dipentaerythritol. The 2.500 figure in the denominator is the weight of original sample.

In an actual test, A was found to be 2.857 and 2.850 grams for separate determinations. Hence, the per cent dipentaerythritol in the sample is:

$$\frac{2.857 - 2.500 + 0.145}{2.5} \times 100 = 20.08\%$$

and

$$\frac{2.850 - 2.500 + 0.145}{2.5} \times 100 = 19.80\%$$

The mixture used in this analysis was made from weighed quantities of pure pentaerythritol and pure dipentaerythritol in the proportions of 80 to 20.

Table I. Determination of Dipentaerythritol

Mixture No.	Known Composition of Original Mixture: Pentaerythritol %	Known Composition of Original Mixture: Dipentaerythritol %	Composition Found by Analysis: Pentaerythritol (by difference) %	Composition Found by Analysis: Dipentaerythritol %
1	99	1	98.6	1.1, 1.4, 1.7, 1.3
			Av.	1.4
2	98	2	98.1	1.6, 2.3, 1.9, 1.8
			Av.	1.9
3	97	3	96.8	3.2, 2.9, 3.5, 3.3
			Av.	3.2
4	93	7	93.1	7.0, 6.5, 7.2, 6.8
			Av.	6.9
5	90	10	90.15	9.85, 9.85
			Av.	9.85
6	80	20	80.07	20.05, 19.80
			Av.	19.93
7	70	30	70.02	29.85, 30.10
			Av.	29.98
8	45	55	45.08	54.92, 54.92
			Av.	54.92
9	40	60	40.03	60.04, 59.90
			Av.	59.97
10	35	65	35.00	64.92, 65.08
			Av.	65.00

REMARKS

An idea of the accuracy and precision of this method can be obtained from Table I.

As it is necessary to have at least 1 part of pure dipentaerythritol for each part of pure pentaerythritol, there is no necessity for using an added amount of dipentaerythritol, when working with mixtures containing more than 50% of dipentaerythritol. In the results listed in Table I, beginning with mixture 8, no dipentaerythritol was added and a 5-gram sample of the mixture was used for analysis instead of 2.5 grams.

Unless the dipentaerythritol used for this method is of the highest purity, serious errors result, particularly in the solubility correction factor.

In extreme cases, a blank using a known mixture of pentaerythritol and dipentaerythritol, having about the same composition as the unknown, should be run simultaneously with the sample under test, in order to establish the solubility correction value for this specific set of conditions.

The importance of uniform technique throughout this method cannot be overemphasized.

The conditions mentioned for the cooling and rewarming to 25° C. must be strictly adhered to, since they have a considerable effect upon the precipitation of the dipentaerythritol.

The crystals which form in the flask should not be permitted to cake to a hard mass, but should be present as relatively fine, individual plates readily removable by means of a stirring rod and a stream of alcohol from a wash bottle.

It is very important that the sample taken for analysis be representative of the mixture under examination. Dipentaerythritol has a very low packing density and a pronounced tendency to segregate when in admixture with the denser and more compact pentaerythritol crystals.

This method has been used on thousands of samples of commercial pentaerythritol products and has, in the hands of skilled technicians, given satisfactory and meaningful results.

ACKNOWLEDGMENT

The author gratefully acknowledges the assistance of many persons on the technical staff of the Trojan Powder Co., particularly of Victor Ginsler, Kenneth Stripp, and E. A. Wernett, in the development of this method.

Measurement and Analysis of Gases in Vacuum-Packed Food Containers

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An apparatus and method for determining the quantity and composition of gases in vacuum-packed and gas-packed food containers are described. The method is applicable to both large and small containers, flexible, semirigid, or rigid. Gas contents as small as 1 cc. may be measured and analyzed with fair accuracy, and gas contents of 5 cc. or more permit accuracy of the order of ±5% or better. Results obtained on a number of vacuum-packed foods, particularly coffee and dry whole milk, are presented, and application of the method to detection of leakage, determination of oxygen and nitrogen permeabilities of packaging materials, and determination of true densities of dry foods is described.

IN THE evaluation of various packaging materials, methods and conditions for vacuum packing, especially of dry or dehydrated food products in flexible containers, means of determining the amount and composition of residual gases in the packages, as well as the changes in these gases, whether through sorption, desorption, generation, permeation, or leakage during storage, have proved of great value. Strachan and Embree (*5*) have described a device and method for sampling and analysis of the gas in large cans of gas-packed dehydrated foods, while a U. S. Department of Agriculture circular (*1*) has described a similar method. Unpublished work is being done in other laboratories on the measurement and analysis of gases in canned food products. All these methods, however, appear to lack the versatility and sensitivity achieved with the author's design of equipment and procedure, especially as applied to small packages containing 113.4 grams (4 ounces) or less of dry food products.

BELL-JAR METHOD OF MEASURING VACUUM

A widely used approximate method for determining the residual vacuum in canned goods is to reduce the external pressure until the cans begin to bulge. This method was readily applicable to flexible packages with considerably greater accuracy because of the much smaller pressure differential required to bulge the flexible package walls noticeably. Essentially, the method consists merely of placing the package under a glass bell jar connected to a vacuum pump and a vacuum gage or a manometer, reducing the pressure in the bell jar to the point at which the first swelling or bulging of the package is visible, and reading the gage or manometer to obtain a direct measure of the residual vacuum or absolute pressure in the package. This has been used as a very rapid method, capable of testing from one to three packages individually per minute, using a small glass bell jar, a dial vacuum gage calibrated in inches of mercury, a 42.5-liters (15 cubic feet) per minute vacuum pump, and quick-acting control and release valves. Comparison of residual vacuum determinations by this method with more reliable values on the same packages obtained by calculation from total residual gas content and gas space volume, as described below, indicates an over-all probable error of not more than ±25.4 millimeters (1 inch), and usually not more that ±0.5 inch, for the rapid bell-jar method, without correction for ordinary variations in room temperature and barometric pressure.

DETERMINATION OF GAS CONTENT OF PACKAGES

Although the amount and composition of gases have been determined in 1-pound cans of vacuum-packed coffee, with gas pressures often greater than 1 atmosphere due to evolution of gases by the coffee after packing, the system used appeared not to be directly applicable to the problem. The author was dealing with packages mostly containing from 3 to 5 ounces of product, and some of the products evolved very little gas after vacuum packing, so that he often had to deal with 1 cc. or less of gas per package. Furthermore, the flexible packaging materials with which he was working, usually laminated sheets such as paper to foil with a heat-sealing inner coating, offered difficulties in the way of attaching a gas-collection system.

After several trial procedures, an apparatus was designed and constructed which not only effectively overcame most of the previous difficulties, but had a number of other useful applications. This apparatus includes a gas-measuring system, with means for connecting a wide variety of hermetically sealed packages to the system in such manner as to sample their gas contents without loss or contamination, and a gas-analysis system. The essential details of the apparatus are illustrated in Figure 1. In practice, the glass parts were made to order of Pyrex and the entire assembly was mounted for convenient manipulation on a specially constructed stand of wood and metal.

The semimicro gas-analysis buret, *G*, is approximately 70 cm. long. It is marked, from the 1-mm. inside diameter capillary tip, by 0.02-cc. divisions along a 5-mm. inside diameter barrel to 10 cc., whence marking continues by 0.2-cc. divisions along a 15-mm. inside diameter barrel to 20 cc. just above the stopcock. This buret is interchangeable with another of similar design and

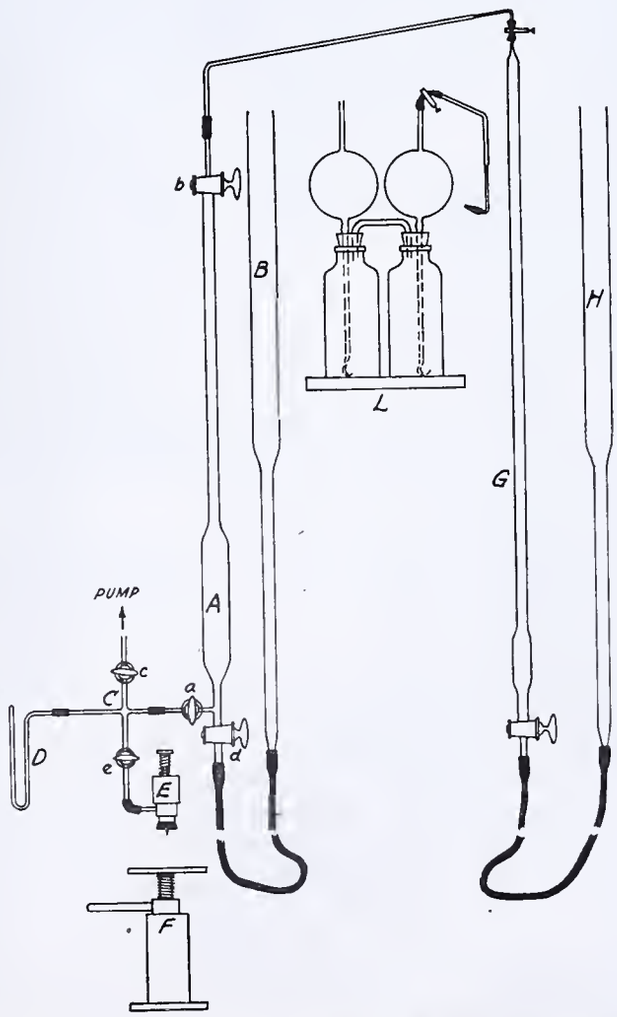

Figure 1. Apparatus for Measuring and Analyzing Gases in Food Packages

the same length, but of 100-cc. capacity, marked by 0.05-cc. divisions from the 1-mm. tip along an 8-mm. inside diameter barrel to 10 cc. and by 0.5-cc. divisions along a 24-mm. inside diameter barrel from there to 100 cc. just above the stopcock. The same leveling tube, *H*, for acidulated water, is used with either buret.

Gas analysis is conducted by means of a number of Richards' pipet assemblies, *L*, with suitable reagents for determination of various gases as desired. The author has used 33% potassium hydroxide solution for carbon dioxide, Fieser's solution for oxygen, and ammoniacal cuprous chloride for carbon monoxide when present, the residue being nitrogen. The specially designed 2-mm. inside diameter transfer tubes, sloping upward and reduced to 1-mm. near their carefully tapered tips, permit alternate connection of the tube from the gas-collection system and those from the several Richards' pipet assemblies to the gas-analysis buret, through a short piece of 2-mm. inside diameter gum rubber tubing fitted with a pinchclamp, without significant loss or contamination of the gas sample or interchange of the reagents in the Richards' pipets with the acidulated water in the buret. Careful operative technique with the equipment described permits determination of the oxygen in a 1-cc. sample of ordinary air with an accuracy of about ±10%, or in a 10-cc. sample with an accuracy of about ±1%.

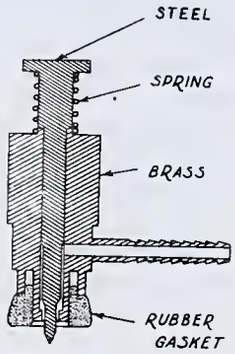

Figure 2. Package-Puncturing Device

The special gas-collection and measuring buret, *A*, has a 2-mm. inside diameter tip above the upper stopcock, is marked by 0.05-cc. divisions from this stopcock along an 8-mm. inside diameter barrel to 10 cc., and by 0.5-cc. divisions along a 24-mm. inside diameter barrel to 100 cc. at a point between the side arm and the lower stopcock, *d*, where the barrel is reduced to 5-mm. The mercury leveling tube, *B*, is similar in size and shape to *H*. The connecting manifold, *C*, and manometer *D* are 2 mm. in inside diameter and as short as convenient. The volume of the capillary system, from stopcock *a* to stopcock *c* to surface of package attached to device *E*, is just 3.0 cc., including the manometer up to its mercury column. All stopcocks are lapped in and lubricated for high-vacuum use, while connections to *C* are made with short pieces of heavy-walled gum rubber tubing, well covered with petrolatum. Air leakage into buret *A* and manifold *C*, through the five stopcocks, three rubber connections, one rubber gasket, and past the shaft of the puncturing pin, does not exceed 0.1 cc. per hour even at full vacuum, which introduces no appreciable error in use of the apparatus.

The package to be tested is supported on the adjustable spring table, *F*, by which it is held firmly against the rubber gasket of the special puncturing device, *E*. This device is shown in section in Figure 2, where the pin is in the depressed or puncturing position. Normally this pin is held up by the spring, so that the tip is retracted well within the metal shell, even against atmospheric pressure, until it is pushed downward into the puncturing position by additional hand pressure on its head. The steel shaft of this pin is carefully machined and lapped into the brass barrel, so that liberal application of petrolatum serves both to lubricate it and to give a vacuum-tight seal. The rubber gasket is also seated and sealed with petrolatum.

The method of sealing the package securely to the puncturing device depends on the type of package. With metal cans, metal-covered glass jars, or firm vacuum packages of flexible material with a smooth, nonporous outer surface, it is sufficient to press the package firmly against the rubber gasket by means of the adjustable spring table, using petrolatum under and around the gasket to complete the seal. With flexible packages of laminated sheet material in which the outer layer is paper, it is necessary to strip the paper carefully away to a nonporous layer of the laminated sheet, such as cellophane or metal foil, taking care not to puncture this layer, and seal this smooth surface to the gasket. When the surface of the package is irregular, as with peanuts vacuum-packed in flexible material, an area slightly larger than the gasket is made level by applying a molten mixture of equal parts of paraffin and petrolatum and allowing it to solidify. Flexible vacuum-packed coffee packages which have become swollen by evolution of gases are prepared by sealing a metal washer to a smooth, nonporous layer of the package material with a molten mixture of paraffin and amorphous wax, then sealing this washer against the rubber gasket with petrolatum in such position that the puncturing pin can pass through the hole in the washer.

The tightness of the seal between the package and the puncturing device is checked, before puncturing the package, by drawing a full vacuum on the 3-cc. capillary system, closing the stopcock to the pump, and observing the manometer. Since leakage of only 0.002 cc. of air into this system raises the pressure by 0.1 mm., even a very slight leak is detected in a few minutes and eliminated by application of more petrolatum around the gasket or puncturing pin.

When the tightness of these seals has been verified, the entire gas-collection system is vacuumized, including the transfer tube to the gas-analysis buret. Then, with the several stopcocks in appropriate positions, the package is punctured by pressing firmly on the head of the puncturing pin, and the gas from the package is allowed to expand into the vacuumized system. If there is very little gas in the package, it is first admitted only to the 3-cc. capillary system, and the equilibrium pressure is read on the manometer. Then it is admitted to the 100-cc. gas-measuring buret, and the manometer is read again. In the absence of complicating factors, such as an equilibrium water-vapor pressure or progressive desorption of gas from the packaged product, these two manometer readings, together with the known volumes of the capillary system and gas buret, permit calculation of the unknown gas-space volume of the package.

The stopcock in the buret side arm is now closed, and the volume of gas in the buret is measured at 1-atmosphere pressure. Part or all of this gas, depending on its amount, is passed through the transfer tube to the gas-analysis buret. It may be held there for subsequent analysis, or successive portions of gas from the same package may be added to it, if necessary to obtain a larger sample for analysis. Each successive portion of gas is withdrawn from the package by lowering the mercury leveling tube until the mercury falls below the side arm, opening the side-arm stopcock to allow the remaining gas to expand into the buret, closing the side-arm stopcock, measuring the gas in the buret at 1 atmosphere, then transferring this gas to the gas-analysis buret, or discarding it, as desired.

GAS MEASUREMENT CALCULATIONS

When dealing with dry powdered or granular packaged materials which do not exhibit significant sorption or desorption of gases, the gas-measurement calculations are relatively simple.

Remembering that the volume of the gas-measuring buret is 100 cc., and of the capillary system 3 cc., let V_0 be the gas-space volume of the package in cubic centimeters, V be the total gas content of the package measured in cubic centimeters at room temperature and 1 atmosphere, P_0 be the total gas pressure in atmospheres in the package before puncturing, and r_1, r_2, r_3- - - be the buret readings as each successive portion of gas from the package is measured at 1 atmosphere. From the simple PV relationship, $V = P_0V_0$, $0.01r_1(V_0 + 103) = P_0V_0$, $0.01r_2(V_0 + 103) = 0.01r_1(V_0 + 3)$, $0.01r_3(V_0 + 103) = 0.01r_2(V_0 + 3)$, etc. It readily follows that $r_1 = 100V/\ V_0 + 103)$, $r_2 = 100V(V_0 + 3)/(V_0 + 103)^2$, $r_3 = 100V \times (V_0 + 3)^2/V_0 + 103)^3$, etc. From this, it is apparent that $r_2/r_1 = r_3/r_2$- - - $r_n/r_{n-1} = (V_0 + 3)/(V_0 + 103) = k$, a constant. Obviously, V is the sum of a geometric series whose first term is r_1 and whose ratio is the constant, k. Therefore, $V = r_1 + r_2$- - - $+ r_n/(1 - k)$, and $V_0 = (103k - 3)/(1 - k)$.

In practice, if there is a substantial amount of gas in the package, and no significant desorption of gas occurs during removal of successive portions, we take the average value of k from the inverse ratios of successive r values, and obtain the values of P_0, V_0, and V from the above relationships. Random variations in successive k values indicate experimental error, while progressive increase in successive k values generally indicates either desorption of gas from the packaged product or a leak in the package or system.

If the amount of gas in the package is very small, or a significant amount of gas is desorbed, a more accurate determination of V_0 is made, after sampling the gas for analysis.

In one case, air is admitted to the package and capillary system to exactly 1 atmosphere, while the buret is vacuumized. Then this air is allowed to expand into the buret, trapped off, and measured at 1 atmosphere as r_a. Again, from the PV relationship, $0.01r_a(V_0 + 103) = V_0 + 3$, or $0.01r_a = (V_0 + 3)/(V_0 + 103) = k$, the constant previously derived. Then, by substitution, $V_0 = (103r_a - 300)/(100 - r_a)$. In the other case, which is generally more convenient, the package and capillary systems are thoroughly pumped out to remove all sorbed gas, then the pump stopcock is closed, and air at 1 atmosphere in the 100-cc. gas-measuring

Table I. Relationship of V_0 to k and to r_r

k	r_r	V_0	k	r_r	V_0	k	r_r	V_0	k	r_r	V_0
0.11	89	9.4	0.31	69	41.9	0.51	49	101	0.71	29	242
0.12	88	10.6	0.32	68	44.1	0.52	48	105	0.72	28	254
0.13	87	11.9	0.33	67	46.3	0.53	47	110	0.73	27	267
0.14	86	13.3	0.34	66	48.5	0.54	46	114	0.74	26	282
0.15	85	14.6	0.35	65	50.8	0.55	45	119	0.75	25	297
0.16	84	16.0	0.36	64	53.2	0.56	44	124	0.76	24	314
0.17	83	17.5	0.37	63	55.7	0.57	43	130	0.77	23	332
0.18	82	19.0	0.38	62	58.3	0.58	42	135	0.78	22	352
0.19	81	20.5	0.39	61	60.9	0.59	41	141	0.79	21	373
0.20	80	22.0	0.40	60	63.7	0.60	40	147	0.80	20	397
0.21	79	23.6	0.41	59	66.5	0.61	39	153	0.81	19	423
0.22	78	25.2	0.42	58	69.4	0.62	38	160	0.82	18	453
0.23	77	26.9	0.43	57	72.4	0.63	37	167	0.83	17	485
0.24	76	28.6	0.44	56	75.6	0.64	36	175	0.84	16	522
0.25	75	30.3	0.45	55	78.8	0.65	35	183	0.85	15	564
0.26	74	32.1	0.46	54	82.2	0.66	34	191	0.86	14	611
0.27	73	34.0	0.47	53	85.7	0.67	33	200	0.87	13	666
0.28	72	35.9	0.48	52	89.3	0.68	32	210	0.88	12	730
0.29	71	37.9	0.49	51	93.1	0.69	31	220	0.89	11	806
0.30	70	39.9	0.50	50	97.0	0.70	30	230	0.90	10	897

uret is allowed to expand into the vacuumized package and apillary system. At equilibrium the side-arm stopcock is closed nd the air remaining in the buret is measured. Calling this eading r_r, we have, from the PV relationship, $100 = 0.01r_r$ $V_0 + 103)$ and $100 - r_r = 0.01r_r(V + 3)$. Dividing the second of hese equations by the first, we find $k = 1 - 0.01r_r$, and, by substitution, $V_0 = (100/0.01r_r) - 103$. Table I gives V_0 for various alues of k and r_r.

DETERMINATION OF TRUE DENSITY

This apparatus permits reasonably accurate determination of he true density of dry powdered or granular materials, such as offee, milk powder, and dehydrated soups, which are not well uited to true density determinations by liquid displacement. n this case, density is actually determined by gas displacement. t is merely necessary to determine the weight of the package ontents and divide this by the total net volume less the gas space olume, to obtain the true density of the packaged product.

In the case of flexible vacuum packages, where there is no free ead space, and the package contents are firmly compressed by external atmospheric pressure, the apparent or bulk density of the product is given by dividing the net weight by the total net volume. There is then, of course, a definite relationship between true density, apparent density, net weight, and gas space volume. When working with a packaged material that is very uniform in apparent and true densities, it is convenient to use this relationship for checking the accuracy of V_0 determinations on individual packages, or indeed as a means of obtaining the most reliable value of V_0 for the individual package.

As an example of this, standard Ottawa test sand was used in work on experimental flexible vacuum packaging to test the effectiveness of different packaging materials and methods. This sand, being substantially pure quartz, has a true density of 2.65 grams per cc. In the 50- to 70-mesh size, found most suitable for this experimental packaging work, its apparent density is consistently 1.62 grams per cc. The gas space volume per gram of packaged sand is then $1/1.62 - 1/2.65 = 0.24$ cc.

ACCURACY OF VOLUME AND PRESSURE DETERMINATION

In addition to experimental errors in reading the buret, other errors may be introduced through failure to level the mercury tube properly, leaks in the system, variations in temperature, failure to establish complete pressure equilibrium before closing the side-arm stopcock, sorption or desorption of water vapor or gases, and in various other ways. However, with careful technique, and by conducting all measurements in a room maintained at 75° ± 2° F., the author has obtained reasonably accurate and reproducible results over a considerable range of gas-space volumes and pressures.

In order to check the accuracy of the method, it has been applied to known conditions from time to time. As an example, a small round-bottomed, ring-necked flask was tightly fitted with a rubber stopper carrying a short capillary connecting tube. The volume was determined, by weight of water contained, as 128.6 cc. This flask, clean and dry, was then connected to the capillary system in place of the special puncturing device, and its gas-space volume was determined repeatedly by the methods described. Typical data for a number of repeat runs, with varying amounts of air in the flask at the start of the run, are given in Table II.

Table II. Gas-Space Volume Results on a Known Volume

Run 1			Run 2			Run 3			Flask Vacuumized, Buret at 1 Atmosphere	
r	k	V_0	r	k	V_0	r	k	V_0	r_r	V_0
55.5			45.0			30.5				
	0.568	129		0.567	128		0.574	132	43.0	130
31.5			25.5			17.5				
	0.572	131		0.569	129		0.560	124	43.5	127
18.0			14.5			9.8				
	0.562	125		0.570	130		0.566	128	53.2	129
10.1			8.25			5.55				
	0.565	127		0.570	130		0.567	128	43.3	128
5.7			4.7			3.15				
	0.570	130		0.564	126		0.572	131	43.2	129
3.25			2.65			1.80				
Av.	0.567	128.2		0.568	128.8		0.568	128.8		128.6

The true density of 50- to 70-mesh Ottawa test sand was found to be 2.65 grams per cc. by displacement of water, in excellent agreement with the literature values of 2.653 to 2.660 grams per cc. for the density of quartz. Then 175.0 grams of this sand, clean and dry, were placed in the flasks described above, and its gas-space volume was again determined. The results are given in Table III.

Table III. True Density of Sand by Gas Displacement

Run 1			Run 2			Run 3			Flask Vacuumized Buret at 1 Atmosphere	
r	k	V_0	r	k	V_0	r	k	V_0	r_r	V_0
38.0			29.0			31.5				
	0.395	62.3		0.397	62.9		0.397	62.9		
15.0			11.5			12.5			60.5	62.3
	0.394	62.0		0.396	62.6		0.396	62.6		
5.90			4.55			4.95			60.3	62.9
	0.398	63.1		0.395	62.3		0.394	62.0		
2.35			1.80			1.95			60.5	62.3
Av.	0.396	62.5		0.396	62.6		0.396	62.5		62.5

True volume of 175.0 grams of sand = 128.6 − 62.5 = 66.1 cc.
True density of sand = 175.0 ÷ 66.1 = 2.65 grams per cc.

Table II shows that V_0 values based on single k values deviated as much as 3% and V_0 values based on single r_r values deviated more than 1% from the known value, but that average determined V_0 values were in excellent agreement with the known value. Deviations of individual V_0 values in Table III from the average are somewhat less, and the true density of sand based on the average V_0 values is in complete agreement with that obtained by displacement of water.

APPLICATION TO FOOD PRODUCTS SEALED IN RIGID CONTAINERS

Although primarily interested in the gas content of flexible vacuum packages, the author has applied the apparatus and

Table IV. Peanuts Vacuum-Packed in a Can

Weights, Grams		Gas Measurement			Gas Analysis	True Density
		r	k	V_0		
Gross	181.6	7.25			Sample, 9.24 cc.	$\frac{125.3}{213-94}$ = 1.05 grams per cc.
Tare	56.3		0.496	95.4	CO_2 1.16 = 12.6%	
Net	125.3	3.60			O_2 0.00 = 0.0%	$P_0 = V/V_0 = 0.156$ atm.
Volumes, Cc.		1.75	0.486	91.6	N_2 8.08 = 87.4%	Vacuum = 25.3 inches
Gross	220.2	Av.	0.491	93.5	Av. V_0 = 94.0	Oxygen reacted with peanuts during storage[a] = 3.4 cc. = 0.025 N.T.P. cc. per gram
Tare	7.2					
Net	213.0	Package Vacuumized, Buret at 1 Atmosphere			$V = \frac{r}{1-k}$ = 14.7 cc.	
Temperature, 75°F.		r = 50.5 cc.			CO_2 = 1.85 cc.	
Barometer, 760 mm.		rV_0 = 95.0 cc.			N_2 = 12.85 cc.	

[a] Assuming all nitrogen present in residue from air sealed in package.

Table V. Whole Milk Powder Gas-Packed in a Can

Weights, Grams		Gas Measurement			Gas Analysis	True Density
		r	k	V_0		
Gross	184.9	21.5			Sample 20.0 cc.	$\frac{124.0}{199.2-93.1}$ = 1.17 grams per cc.
Tare	60.9		0.490	93.1	CO_2 17.0 = 85.0%	
Net	124.0	10.5			O_2 0.08 = 0.4%	$P_0 = V/V_0 = 0.454$ atm.
Volumes, Cc.		5.20[a]	0.495[a]	95.0[a]	N_2 2.92 = 14.6%	Vacuum = 16.4 inches
Gross	207.0	2.70[a]	0.520[a]	105[a]	Av. V_0 = 93.1 cc.	Oxygen reacted with milk during storage[b] = 1.47 cc. = 0.011 N.T.P. cc. per gram
Tare	7.8					
Net	199.2	Package Vacuumized, Buret at 1 Atmosphere			$V = \frac{r_1}{1-k}$ = 42.2 cc.	
Temperature, 75° F.		r_r = 51.0 cc.			CO_2 = 35.87 cc.	
Barometer, 761 mm.		V_0 = 93.1 cc.			O_2 = 0.17 cc.	
					N_2 = 6.16 cc.	

[a] These values, high due to desorption of gas, omitted from averages.
[b] Assuming all nitrogen present is residue from air sealed in package.

method to food products packed in various sizes of cans and glass jars with metal or paperboard covers. The usual procedure with such packages is to weigh them and determine their gross volume by displacement of water, then to seal them to the apparatus and determine the gas space volume and gas content and composition, and finally to empty the contents and determine the tare weight and volume. The true density of the solid contents may be calculated from these data. The results obtained on a vacuum-packed can of peanuts are given in Table IV.

Examination of the data in Table IV indicates that the package was free from leaks, that there was little desorption of gas from the peanuts during examination, that the peanuts were relatively freshly roasted when packed, since they evolved some carbon dioxide after packing, that they were sealed at approximately 24.8-inch vacuum, and that during storage substantially all the oxygen of the residual air in the package reacted with the peanuts. The age of this package of peanuts was not known, but it was at least several months. The peanuts were crisp and dry, but very slightly stale and rancid, owing to the action of the oxygen absorbed.

The results obtained on a can of commercially gas-packed whole milk powder purchased on the open market are given in Table V.

The fact that the true density found here is lower than the 1.27 value reported by Muers and Anderson (*4*) is attributed to effectively sealed gas spaces inside the particles, and indicates 0.07 cc. of such contained gas per gram of powder, in agreement with the values which they reported for normal whole milk powders. When the milk from this package was reconstituted to normal whole milk concentration, it was found to be very slightly rancid. This would be expected from the indicated amount of oxygen in the package when sealed, which is slightly in excess of the maximum safe limit of 0.01 cc. per gram reported by Lea, Moran, and Smith (*3*).

Although the apparatus was primarily designed for examination of smaller packages, it has been applied satisfactorily to 1-pound cans of vacuum-packed coffee. Table VI presents a typical set of results on this type of package examined 25 days after packing.

Here we have an example of a semirigid container which is swollen somewhat at the start of the test, because of an internal gas pressure greater than 1 atmosphere, and of a packaged product which progressively desorbs gas as the pressure is reduced, both conditions complicating interpretation of the data and making the results less certain. However, results on a number of similar packages on the same lot of coffee were in agreement within ±5% or better.

APPLICATION TO FOOD PRODUCTS IN FLEXIBLE VACUUM PACKAGES

The method has been applied to large numbers of flexible vacuum packages of dry food products, including coffee, whole milk powder, ice cream mix, dehydrated soups, and peanuts, generally obtaining agreement within ±5% or better on duplicate packages of the same lot.

To illustrate results on a flexible package with above 1 atmosphere internal gas pressure, the data in Table VII are for freshly roasted drip-grind coffee, of the same lot as that in Table VI, but vacuum-packed in a nonpermeable flexible package 1.5 hours after grinding, and examined 19 days after packing.

The figures for true density of this sample of drip-grind coffee are in close agreement when packed in the rigid and in the flexible package, as shown in Table VI and VII.

Application of the method to flexible vacuum packages with very little residual gas is illustrated in Table VIII, covering examination of a package of dry whole milk, sealed at 29.5-inch vacuum in a nonpermeable flexible package, and examined 6 weeks later.

The calculation of gas contained in the dry milk particles is based on the true density of 1.27 grams per cc. reported by Muers and Anderson (*3*) and the value of 1.14 found by gas displacement. This amounts to 9.4 cc. for the 105 grams of dry milk in that package, and offers an explanation of the source of the 6.0 cc. of air found in the package, as compared with less than 2 cc. that should have been left in the free gas space of the package when it was sealed at 29.5-inch vacuum. This package, and others of the same lot, showed a bell-jar vacuum of 29.5 inches immediately after sealing, dropped to 29 inches overnight, and reached substantial equilibrium at 27 inches in about a week. This is clear evidence that the increasing internal gas pressure was not due to leakage, but to gradual desorption of gas from the milk powder.

Apparently, air contained inside the walls of the dry milk particles was not entirely removed during vacuumizing and sealing of the package, but gradually diffused into the space between the particles after the package was sealed, until equilibrium was established.

Among the many studies of gas content of food products in flexible vacuum packages to which the method has been applied, an interesting example is that of the relationship between fineness of grind and time between grinding and packing of freshly roasted coffee and the amount and composition of gas evolved by the vacuum-packed coffee at equilibrium.

A representative 15-pound sample of a regular commercial blend of Colombian and Costa Rican coffee was taken from a 450-pound lot immediately after roasting. Twenty minutes later, this sample was ground on a small power grinder, one third of it to "regular grind" size, one third to "drip-grind" size, and one third to "pulverized" size. These three grinds were then packed at 29.5-inch vacuum in lots of five packages of each grind, at four different time intervals after grinding. Bell-jar vacuum determinations were made on these packages at intervals for 40 days, by which time substantial pressure equilibrium had been reached. The packages were then examined for gas content. The bell-jar vacuum results are given in Table IX, each value representing the average for five packages.

The results of the examination of these packages for gas content and gas composition are summarized in Table X, each value representing the average for five packages. In general, results for individual packages did not deviate from the group averages by more than ±2 to ±5%.

Results such as those in Tables X and X, for a particular blend and roast of coffee, permit selection of fineness of grind and time interval between grinding and packing required to prevent swelling of flexible vacuum packages due to gases evolved after sealing. They also show the amount of residual oxygen in the packages, which eventually reacts substantially completely with the coffee. Studies are planned to correlate the amount of absorbed oxygen with the staleness or rancidity of the coffee after various times and conditions of storage.

Approximate determinations of true density were made on freshly roasted and ground coffee by displacement of a light petroleum naphtha, but some error due to solubility of the coffee oil is introduced. The results vary for different grinds of the same coffee, increasing with the fineness of grind, indicating substantial residual entrapment of gases in the cellular structure. The author has found true density values for coffee varying from 0.90 gram per cc. for a lightly roasted regular grind to 1.30 grams per cc. for a heavily roasted pulverized coffee. These figures check those shown in Table X, obtained by a different method.

Table VI. Drip-Grind Coffee Vacuum-Packed in a Can

Weights, Grams	Gas Measurement	Gas Analysis	True Density
Gross 606.5 Tare 151.5 Net 455.0	r 90.0 k 0.839 V_0 518[a] r 75.5 k 0.841 V_0 526[a]	Sample 50.0 cc. CO_2 40.5 = 81.0% O_2 0.45 = 0.9% CO 3.30 = 6.6% N_2 5.75 = 11.5%	$\frac{455}{990-510}$ = 0.95 gram per cc.
Volumes, Cc.	r 63.5	$V = \frac{r}{1-k}$ = 550 cc.	$P_0 = V/V_0$ = 1.08 atmospheres
Gross 1010 Tare 20 Net 990	Package Vacuumized, Buret at 1 Atmosphere	CO_2 = 445 cc. CO = 36 cc. O_2 = 5 cc. N_2 = 63 cc.	Vacuum = −2.4 inches Oxygen reacted with coffee during storage = 12 cc. = 0.024 N.T.P. cc. per gram
Temperature, 75° F. Barometer, 762 mm.	r_r = 17.0 cc. V_0 = 485 cc.[b] Probable V_0 = 510 cc.[c]		

[a] Values somewhat high, owing to progressive desorption of gas.
[b] Value low, because ends of can are now pushed in somewhat.
[c] Approximate value at start of test, probably ±5 to 10 cc.

Table VII. Drip-Grind Coffee Vacuum-Packed in a Flexible Package

Weights, Grams	Gas Measurement	Gas Content	Density
Gross 92.2 Tare 6.8 Net 85.4	r_1 = 53.5 cc. r_r = 55.5 cc. V_0 = 77.2 cc.[a]	$V = \frac{r_1(V_0 + 103)}{100}$ = 96.5 cc. CO_2 = 85.1 cc. CO = 6.2 cc. O_2 = 0.3 cc. N_2 = 4.9 cc. Oxygen reacted with coffee during storage = 1.0 cc. = 0.011 N.T.P. cc. per gram $P_0 = V/V_0$ = 1.25 atmosphere[c]	True = $\frac{85.4}{170-77.2}$ = 0.92 gram per cc. Bulk = 85.4/170 = 0.50 gram per cc.
Volumes, Cc. Gross 175[b] Tare 5 Net 170	Gas Analysis Sample 50.0 cc. CO_2 44.1 = 88.2% O_2 0.15 = 0.3% CO 3.20 = 6.4% N_2 2.55 = 5.1%		
Temperature, 75° F. Barometer, 758 mm.			

[a] With package walls collapsed against contents.
[b] From measurement of package dimensions before evolution of gas caused it to swell.
[c] If package walls had not swelled.

Table VIII. Dry Whole Milk in a Flexible Vacuum Package

Weights, Grams	Gas Measurement	Gas Analysis	Density
Gross 112.0 Tare 7.0 Net 105.0	r 3.75 k 0.374 V_0 56.7 r 1.40 r_r = 62.0 cc. V_0 = 58.3 cc.	Sample 5.00 cc. O_2 1.00 = 20.0% N_2 4.00 = 80.0%	True = $\frac{105}{150-57.5}$ = 1.14 grams per cc.
Volumes, Cc. Gross 155 Tare 5 Net 150	Av. V_0 = 57.5 cc.	$V = \frac{r_1}{1-k}$ = 6.0 cc. O_2 = 1.2 cc. N_2 = 4.8 cc. $P_0 = V/V_0$ = 0.103 atmosphere	Bulk = 105/150 = 0.70 gram per cc. Gas contained in dry milk particles = 1/1.14−1/1.27 = 0.09 cc. per gram
Bell-jar vacuum = 27 inches			Vacuum = 26.9 inches

Table IX. Effect of Grind and Age on Gas Pressure Developed by Coffee

Average bell-jar vacuum readings for groups of five 3-ounce flexible vacuum packages of one lot of freshly roasted coffee, in three grinds, packed at four times after grinding

Days after Packing	Age When Packed, Hours											
	Regular Grind				Drip Grind				Pulverized Grind			
	0.6	1.3	3.5	4.1	0.6	1.3	3.5	4.1	0.6	1.3	3.5	4.1
	Inches				*Inches*				*Inches*			
1	6	10	14	15	7	11	15	16	26	26	26	26
2	2	6	11	12	4	8	12	13	25.5	25.5	25.5	25.5
4	−1	2	7	8	1	5	8	9	25	25	25	25
8	−2	0	4	5	0	3	6	7	24	24	24	24
14	−4	−2	1	2	−2	0	2	3	23	23	23	23
40	−5	−3	0	1	−3	−1	1	2	22	22	22	22

Negative values represent internal pressures above 1 atmosphere, and are only estimates of conditions that would have prevailed if package walls had not swelled.

APPLICATION TO GAS LEAKAGE OR PERMEATION INTO PACKAGES

Leakage or permeation of air into flexible vacuum packages made of various sheet materials can be determined by this method. Test packages are filled with the 50- to 70-mesh Ottawa sand previously described and sealed at 29.5-inch vacuum. These packages are then stored under any desired conditions and tested from time to time for residual vacuum by the bell-jar method. Representative packages are examined at suitable intervals for gas content and gas composition. Since the sand exhibits no significant sorption or desorption of gases, any change in gas content on storage is due to leakage through pinholes or faulty seals or to permeation through the sheet material itself.

In general, uniform decrease in residual vacuum for all similar packages made of a particular material is clear evidence of gas entry by permeation instead of leakage, since it is extremely improbable that any two packages would leak at exactly the same rate. When gas entry is by permeation only, determination and

Table X. Effect of Grind and Age When Packed on Gas Evolved in 40 Days by Coffee Vacuum-Packed in Impermeable Flexible Packages

	Regular Grind				Drip Grind				Pulverized Grind			
Av. true density, grams per cc.	0.95				1.00				1.30			
Av. bulk density, grams per cc.	0.50				0.52				0.60			
Age when packed, hours	0.6	1.3	3.5	4.1	0.6	1.3	3.5	4.1	0.6	1.3	3.5	4.1
Av. net weight, grams	78.0	76.8	81.6	80.2	81.0	79.2	80.6	80.8	77.0	77.0	81.0	86.8
Av. gas space, V_0, cc.[a]	74	73	77	76	75	73	75	75	70	69	73	78
Av. gas content, V, cc.[b]	135	115	90	80	115	90	80	75	19.5	19.0	19.5	20.5
Av. pressure, P_0, atm.[c]	1.82	1.58	1.17	1.05	1.54	1.23	1.07	1.00	0.278	0.275	0.267	0.263
Av. gas composition												
CO_2, cc.	125	105	80	70	106	81	71	66.2	12.5	12.0	12.0	12.6
CO, cc.	6.3	6.0	5.8	5.7	5.5	5.6	5.5	5.4	3.0	2.8	3.0	3.2
O_2, cc.	0.5	0.6	0.5	0.4	0.4	0.3	0.3	0.2	0.2	0.2	0.2	0.2
N_2, cc.	3.2	3.4	3.7	3.9	3.1	3.1	3.2	3.2	3.8	4.0	4.3	4.5
Av. CO_2, N.T.P. cc. per gram	1.47	1.26	0.90	0.80	1.20	0.94	0.81	0.75	0.15	0.14	0.13	0.13

[a] Calculated from average true and bulk densities, independently determined.
[b] Calculated by formula $V = r_1 (V_0 + 103)/100$.
[c] From $P_0 = V/V_0$; values over 1.00 are those that would obtain if package walls were rigid.

Table XI. Sand Vacuum-Packed in Permeable Flexible Packages

Package No.	1	2	3	4	5	6	7	8	Av.
Net weight, grams	265	275	276	286	271	293	269	277	277
Gas space, V_0, cc.	63.6	66.0	66.3	68.6	65.0	70.4	64.6	66.5	66.5
Day examined, D	0	0	10	10	42	42	98	98	
O_2 pressure, P, atmosphere	0.002	0.002	0.063	0.064	0.163	0.152	0.201	0.202	
N_2 pressure, P, atmosphere	0.008	0.007	0.051	0.052	0.166	0.152	0.316	0.307	
O_2 permeability, R_0, N.T.P. cc. per sq. meter per day per atmosphere			71	74	71	66	64	68	69
N_2 permeability, R_N, N.T.P. cc. per sq. meter per day per atmosphere			12	12	11	10	10	10	11
Air permeability, R_A, N.T.P. cc. per sq. meter per per day per atmosphere, calculated from O_2 and N_2 permeabilities			24	25	24	22	21	22	23
Directly determined on sheet material									27

analysis of the gas in the package permit calculation of the permeability of the package material to the surrounding gas or gases during storage. In work thus far, the author has used only storage in air at 75° F., 50% relative humidity, ambient atmospheric pressure, which has permitted determination of the permeability of various packaging materials to oxygen and to nitrogen under these conditions. The method, however, should be equally useful for determining permeability to other gases under other temperature and humidity conditions by suitable alteration of the storage conditions.

For gas entering a package by permeation, with no sorption or desorption by the package contents, $dV = 1.087\ RA\ (P_x - P)dD$, where V is the volume of gas inside the package, if measured in cubic centimeters at 75° F., 1 atmosphere, R is the permeability of the package material in N.T.P. cc. per square meter per day for 1-atmosphere pressure difference, A is the area of the package material in square meters, P_x is the external gas pressure in atmospheres, P is the internal gas pressure in atmospheres, and D is time in days. But, since $V = PV_0$, where V_0 is the gas-space volume of the package in cubic centimeters, $dV = V_0 dP$; whence we have $\ln(P_x - P_1) - \ln(P_x - P_2) = 1.087\ RA\ (D_2 - D_1)/V_0$.

This relation, which holds for each gas independently of the other gases present, permits calculation of the permeability of the package material from data on the gas content of the package, obtained by the author's method a suitable number of days after packing. Conversely, when the permeability of the package material to oxygen and to nitrogen is known, it permits calculation of air permeation into the package and prediction of internal pressure at any future date.

Most test packages of sand for this work have been of one standard size, containing approximately 300 grams of sand, where $A = 0.030$ square meters and V_0 is approximately 72 cc. The actual value of V_0 for these packages is 0.24 times the net weight of sand, as shown above. When these packages are sealed at 29.5-inch vacuum, the residual air left in each package is approximately 1.4 cc., or 0.3 cc. of oxygen and 1.1 cc. of nitrogen, so that for oxygen $P_1 = 0.004$ and $P_x = 0.21$, while for nitrogen $P_1 = 0.015$ and $P_x = 0.79$.

In studying the properties of a given packaging material, a considerable number of test packages are filled with sand and sealed at 29.5-inch vacuum. All are checked for uniformity of residual vacuum by the bell-jar method, which incidentally serves to detect any that are leaking moderately. A few representative packages are examined for amount and composition of gas, to establish the P_1 of oxygen and nitrogen at time D_1. The remaining packages are checked for uniformity of decrease in residual vacuum at suitable intervals. When there has been a substantial drop in vacuum, say to 25 inches or so, one or more packages are examined for amount and composition of gas. This permits calculation of the permeability of the package material to oxygen and nitrogen and prediction of the amounts of each of these gases in the remaining packages as of any future date.

As an illustration of the application of this method data are given in Table XI on eight packages of sand packed at 29.8-inch vacuum in a cellulose acetate–Pliofilm lamination.

The values of air permeability hold only for the condition that the ratio of partial pressure of oxygen to partial pressure of nitrogen is the same on both sides of the sheet as their ratio in normal air. The calculated air permeability is given by $R_A = 0.21\ R_0 + 0.79\ R_N$, while the direct determination of air permeability was by a method developed by the author (*2*).

Similar determinations on various cellophane-Pliofilm laminations have shown oxygen permeabilities ranging from 6 to 30 and nitrogen permeabilities from 2 to 10 N.T.P. cc. per square meter per day per atmosphere. With the exception of one experimental transparent sheet material which, in a limited preliminary study, has shown an oxygen permeability of less than 1 N.T.P. cc. per square meter per day per atmosphere, these are the lowest permeabilities the author has found for flexible packaging materials containing no metal foil. On the other hand, a considerable number of materials containing 0.0005 inch or thinner aluminum foil in combination with other materials, including cellophane, Pliofilm, and various heat-sealing coatings, have shown no measurable gas permeability.

The maximum permissible gas permeability of sheet material used for flexible vacuum packaging depends, of course, on the product to be packaged and the shelf life required of the package. If it is merely required that the package retain a substantially lower pressure than atmospheric for a few months, a material such as cellulose acetate–Pliofilm lamination might serve. However, for many food products, protection against oxygen is of utmost importance and is the primary reason for resorting to

vacuum packaging. In these cases, the package material must be substantially impermeable to oxygen and to water vapor in many instances.

Dry whole milk, for example, must not be permitted to absorb more than some 0.04 cc. of oxygen per gram (4) if it is to remain free from staleness or rancidity. Only about 10% of this amount remains in the package when it is vacuum-packed under the most favorable commercial conditions. A 4-ounce flexible vacuum package, with a total sheet material area of 0.03 square meter and an oxygen permeability of 1 N.T.P. cc. per square meter per day per atmosphere, would admit 0.007 cc. of oxygen per day, reaching the maximum permissible limit of 4 cc. in about 18 to 20 months, and might be considered to give commercially acceptable protection against oxygen. At the same time, dry whole milk should not be allowed to gain more than some 2% in moisture content during storage, indicating a maximum permissible water vapor permeability of 0.13 gram per square meter per day for the sheet material, under the conditions of storage, to give adequate protection for 18 to 20 months. Incidentally, in this case the rates of permeation of both oxygen and water vapor into the package would remain practically constant, not falling off logarithmically with time, because both would be absorbed substantially completely by the dry milk instead of building up any appreciable back pressure.

ACKNOWLEDGMENT

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Determination of Egg Yolk in Egg White

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A method for the quantitative determination of traces of egg yolk in white involves the extraction and estimation of the cholesterol carried into the whites by the contaminating yolks, and the correlation of this value with the yolk content. Data are presented establishing the constancy of this relationship.

IN THE production of bulk egg whites problems of variation in the finished product may arise to which there are no ready answers. The quality of a batch of egg whites has generally been estimated by functional bakery tests which include measurement of the whipping properties of the whites together with examination of a test cake. These tests serve to detect poor quality, but give no clue as to its cause. Possible causes may include unknown quality characteristics, conditions of storage, or the presence of yolk in the white. It is this last item with which this paper deals.

During the breaking and separating process it is possible for a small amount of yolk to escape into the white, and bakery tests have shown that only a trace of yolk is needed to reduce materially the quality and performance of the white. In fact, as little as 0.05% yolk in the white leads to a noticeable lowering in quality, while the presence of 0.10% yolk may result in a product which is below a satisfactory standard.

The quantitative determination of minute quantities of a material as complex in composition as egg yolk necessarily involves the use of procedures not generally encountered in routine analysis. The obvious attack is to select some constituent present in the yolk but not present in the white, and set up a procedure for its determination. To be of much value, this constituent must bear a close correlation to the actual amount of yolk present—i.e., it must occur in approximately the same percentage in all egg yolks.

Since egg white contains no fat, the first approach to the problem was an attempt to extract the fat present in yolk-contaminated whites, correlating this result with the percentage of fat in yolk. These efforts were unsuccessful because of the extremely small quantity of fat actually contributed by a trace of yolk and difficulty in extracting this small amount. Attention was then turned to the possibility of detecting the cholesterol carried into the whites by the contaminating yolks.

Cholesterol in trace quantities has been detected by colorimetric means for many years and much has appeared in the literature, especially in the field of blood chemistry. The problem at hand appeared to be that of quantitatively isolating the cholesterol from the contaminated white. Procedures of this type have been applied to other dairy products including ice cream, one such procedure being the Mojonnier modification of the Roese-Gottlieb method used by Lampert (2) in his work on ice cream mixtures. This procedure served as a basis for a beginning, but several important changes were necessary in order to obtain quantitative extraction, since the original method was intended for use on mixtures containing much higher percentages of yolk than those encountered here. Further modifications were necessary because egg white, in the presence of alcohol, one of the solvents used in the method, coagulates into a gelatinous mass which resists extraction.

The method used to develop the color was based on the Liebermann-Burchard reaction for cholesterol. Many references to this method appear in the literature and the results reported are numerous and varied. The procedure is essentially as follows:

Extracted cholesterol is dissolved in some anhydrous reagent such as chloroform. An aliquot is pipetted into a dry tube and a specific volume of a 10 to 1 mixture of acetic anhydride–sulfuric acid reagent is added. The color which develops is matched with that obtained with standards treated in the same manner, prepared from pure cholesterol dissolved in the same solvent.

The Coleman Universal Model 11 spectrophotometer with PC-4 filter was used throughout this work, and round cuvettes, 19 × 105 mm., were used for all readings. To establish ideal conditions it became necessary to study color development of this reaction.

EXPERIMENTAL

The first efforts were directed toward applying the Coleman spectrophotometer to reading the color developed by the Liebermann-Burchard reaction. Solutions containing pure cholesterol in chloroform were prepared and a procedure recommended by Reinhold (3) for color development was used. Five milliliters of

a chloroform solution containing pure cholesterol were pipetted into a dry test tube, 2 ml. of a 10 to 1 mixture of freshly distilled acetic anhydride and sulfuric acid were added, and the solution was allowed to stand in the dark at 35° C. for 24 minutes. The green color which developed was read in the spectrophotometer against pure chloroform in the solvent cell. The transmittance was determined over the range 400 to 800 mμ using the appropriate filters. The spectral transmittance curve obtained appears in Figure 1. The point of maximum absorption, 640 mμ, was used for all measurements.

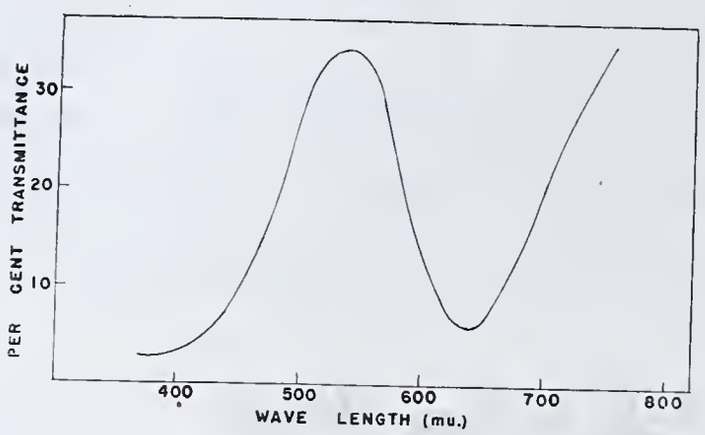

Figure 1. Spectral Transmittance Curve

Color developed with pure cholesterol in chloroform using acetic anhydride–sulfuric acid mix

The intensity of the color produced by the Liebermann-Burchard reaction is dependent not only upon the cholesterol content, but also upon the time of standing and the temperature. A study was made of these factors to establish the most suitable conditions. When color development was allowed to take place at 35° C., the color increased sharply in intensity during the first few minutes of reaction, after which it gradually faded to a much lighter shade.

Work by Ireland (*1*) indicated that use of lower temperatures during color development leads to much more favorable results. After trying several temperatures, it was found that color development at 18° C. in the dark gave the best results. A comparison of the rate of color formation at 35° and 18° C. appears in Figure 2.

While color development is slower at 18° C., it leads to conditions of higher stability near the point of maximum intensity; the change in transmittance between readings at the 20- and 30-minute intervals is 0.5%. This eliminates the need for making readings after an exact time has elapsed and permits an analyst to run a greater number of samples at one time. The curve in Figure 2 indicates that the color near the point of maximum intensity, when developed at 35° C., is unsatisfactory, as it remains at this intensity only momentarily and is in a gradual state of change thereafter. After standing for 25 minutes the rate of change is not so rapid, extensive fading having already occurred. Transmittance curves for known amounts of cholesterol subjected to the color development procedure at both temperatures are shown in Figure 3.

As indicated in the graph, the lower temperature is the more favorable. Standing time refers to standing in the lightproof cabinet until immediately before reading.

For ordinary work a suitable container can be made by wrapping several sheets of asbestos paper around a tin can. The lid is lined with asbestos with a small hole through the top just large enough to permit a thermometer to pass. The can may be placed on an asbestos pad. Water is poured into the cabinet and regulated to 18° ± 0.5° C. It will be found to change less than 0.5° C. in a half hour. The tubes containing the chloroform solution of cholesterol must remain in the bath at 18° C. until they come to temperature. The bath is regulated to maintain proper temperature control and the reagent is then delivered to each tube. The lid is replaced until the time for development has elapsed.

Some difficulty was encountered in extracting the cholesterol by the Mojonnier modification of the Roese-Gottlieb procedure which calls for successive additions of alcohol, ether, and petroleum ether, with a shaking period after each addition. The alcohol coagulated the egg white to form a gelatinous mass which resisted extraction. After several unsuccessful modifications it was found that addition of 25 ml. of ammonia to the sample before introducing the solvents left it in a highly fluid state. Three extractions are made, but the ammonia need be added only once. This procedure is set up to determine all the cholesterol present in a 10-gram sample of egg white, but when analyzing yolk, a 1-gram sample is used and the extracted cholesterol is dissolved in chloroform. This is made to volume in a 250-ml. volumetric flask and a 5-ml. aliquot is used for analysis.

In order to establish the cholesterol content of egg yolk, numerous individual yolks were analyzed. Some of the values obtained appear in Table I.

These data confirm results reported in the literature and lie in a sufficiently close range to provide useful criteria for the amount of yolk present.

Eggs of varying histories were separated and the uncontaminated whites were analyzed (Table II) to determine the "blank" value which would be obtained when no yolk was present.

Table I. Cholesterol Content of Egg Yolk

Yolk No.	Cholesterol	Cholesterol Contributed by Adding 0.05% Yolk to 10 Grams of White
	%	*Gram*
1	1.30	0.000065
2	1.20	0.000060
3	1.35	0.000067
4	1.35	0.000067
5	1.45	0.000072
6	1.20	0.000060
7	1.35	0.000067
8	1.25	0.000062
9	1.30	0.000065
10	1.40	0.000070
Av.	1.32	0.000065

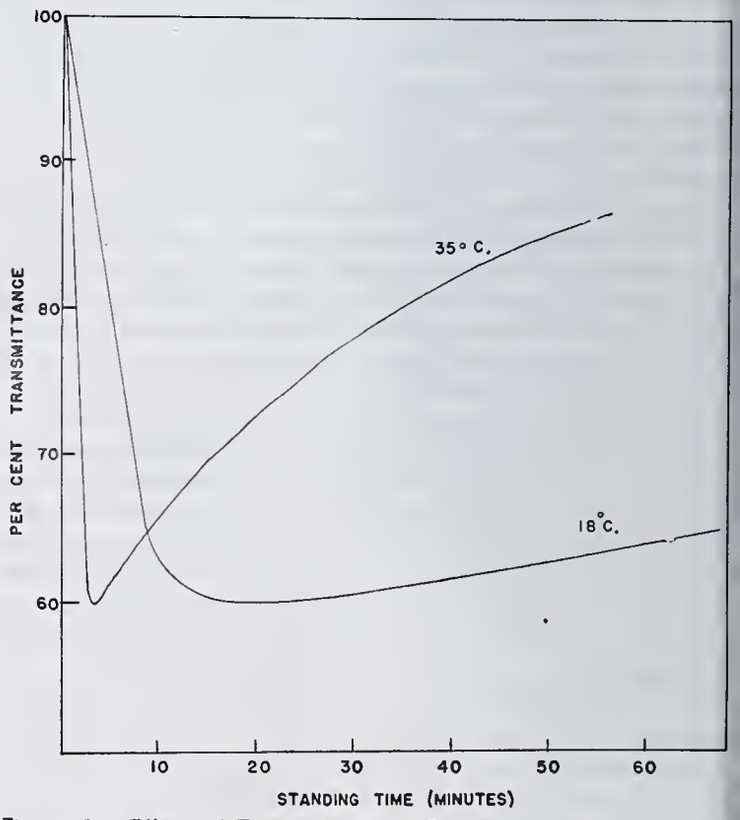

Figure 2. Effect of Temperature on Transmittance over a One-Hour Standing Period

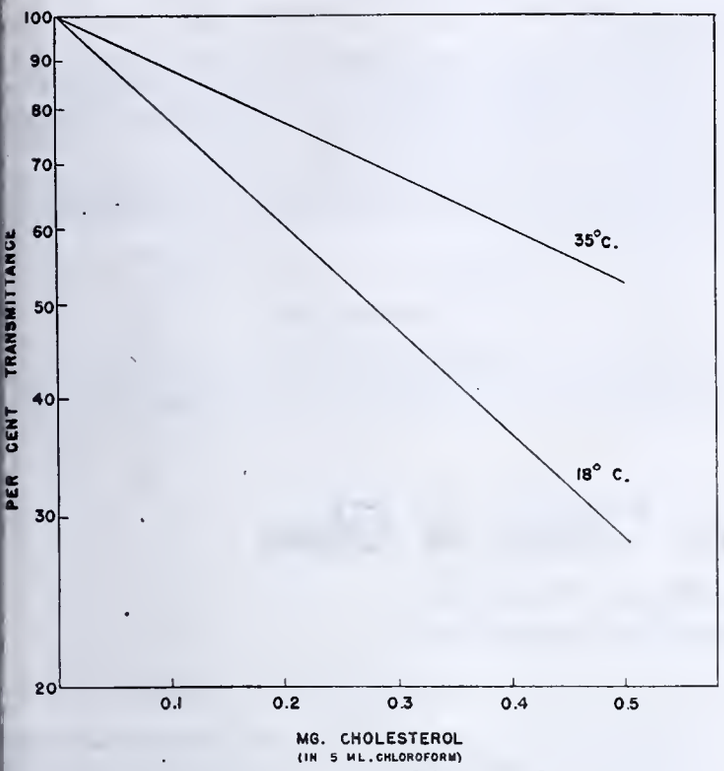

Figure 3. Comparison of Calibration Curves

At 35° and 18° C., 650 mμ

Table II. Blank Value of Pure Egg White

White	Cholesterol in 10 Grams of Sample, *Gram*
Fresh white	0.00011
	0.00011
	0.00011
	0.00011
	0.00012
	0.00011
	0.00012
	0.00011
White from 6 months old cold storage egg	0.00012
	0.00012
Av.	0.000114

This blank value appears to be essentially the same for all egg whites. Analyses of a large number of samples of pure egg whites confirm the constancy of this figure.

Having established the cholesterol content of yolk and the blank obtained with pure white, mixtures of yolk in white in varying percentages were prepared and analyzed.

Table III indicates that the process accounts for all the cholesterol in the sample. Agreement between actual figures and theoretical values is extremely close. On the basis of these findings it is possible to set up a table relating the weight of cholesterol found to the per cent yolk responsible for its presence (Table IV).

A reagent blank determination should also be made by adding the total amount of solvents used for the analysis of one sample to a beaker and evaporating them on the steam bath. The residue should be carried through all the steps in the analysis and the value obtained subtracted from that found for the sample. Occasional contamination of solvents will lead to high results if a correction is not made.

METHOD

Reagents. Ammonium hydroxide (sp. gr. 0.90), A.C.S. specification.

Ethyl Alcohol (95%).

Ethyl ether, A.C.S. specification.

Petroleum ether, b.p. 35–38° C., A.C.S. specification.

Potassium hydroxide, A.C.S. specification.

Chloroform, A.C.S. specification.

Cholesterol, powdered, reagent quality.

Table III. Analysis of Egg Whites Containing Known Amounts of Added Yolk

Sample	Cholesterol in 10 Grams of Sample, *Gram*	Cholesterol Theoretically Present in 10 Grams of Sample, *Gram*
Pure white + 0.05% yolk	0.00018	0.00018
	0.00018	
	0.00018	
	0.00018	
Av.	0.00018	
Pure white + 0.10% yolk	0.00024	0.00024
	0.00023	
	0.00024	
	0.00023	
	0.00024	
Av.	0.00024	
Pure white + 0.20% yolk	0.00037	0.00037
	0.00035	
	0.00036	
	0.00037	
Av.	0.00036	

Sulfuric acid (sp. gr. 1.84), A.C.S. specification.

Acetic anhydride, A.C.S. specification.

Solutions. Acetic anhydride–sulfuric acid mixture. Add 10 ml. of sulfuric acid (sp. gr. 1.84) slowly and with cooling to 100 ml. of freshly distilled acetic anhydride in a 200-ml. glass-stoppered Erlenmeyer flask. Reagent should be water-white.

Preparation of Standard Curve. Weigh accurately 50 ± 0.2 mg. of pure cholesterol into a clean dry 500-ml. volumetric flask. Add pure chloroform to dissolve and make to volume with chloroform. This solution contains 0.1 mg. of cholesterol per ml.

Pipet 1, 2, 3, 4, and 5 ml. of the cholesterol solution into five clean dry 15 × 150 mm. test tubes, and make total volume of each to 5 ml. with chloroform. Place in a lightproof constant-temperature bath at 18° C. for 5 minutes. Check temperature of bath at the end of 5-minute period and readjust to 18° C. if necessary.

Add 2 ml. of acetic anhydride–sulfuric acid mixture (18° C.) to each tube, mixing each thoroughly with a clean, dry, glass rod. Do this as quickly as possible, allowing a minimum of light to enter the cabinet. Replace cover on cabinet and allow to stand for 25 minutes.

Leave each tube in the cabinet until immediately before reading. Using an appropriate filter determine the transmittance in a spectrophotometer set at 640 mμ with a solvent cell of pure chloroform set at 100% transmittance. Plot transmittance against concentration of cholesterol using semilogarithmic paper. This is the standard curve.

Procedure. Weigh 10 ± 0.1 grams of liquid egg white into a 200-ml. glass-stoppered flask, add 25 ml. of ammonium hydroxide (sp. gr. 0.90), and swirl to mix. Add 10 ml. of 95% alcohol and shake for 1 minute. Add 25 ml. of ethyl ether and shake for 1 minute. Add 25 ml. of petroleum ether and shake for 1 minute. Allow to stand until the clear solvent layer separates at the top. If an emulsion forms, break it with 5 ml. of alcohol.

Decant the clear solvent layer into a clean, dry, 250-ml. beaker. Repeat the extractions on the residue in the flask twice, beginning with the addition of alcohol, but using 5 ml. of alcohol each time instead of 10, adding each final solvent layer to the 250-ml. beaker.

Place the beaker containing the extracts on a steam bath and evaporate until only a small amount of alcohol remains. Remove and cool. Add 15 ml. of alcohol, cover with a watch glass, and bring to a boil on a hot plate covered with a sheet of asbestos.

Table IV. Relationship between Cholesterol Content and Egg Yolk Present

Cholesterol Found in 10 Grams of White	Egg Yolk Present, %
0.00011	0.000
0.00015	0.025
0.00018	0.050
0.00021	0.075
0.00024	0.100
0.00027	0.125
0.00030	0.150
0.00033	0.175
0.00037	0.200

Add 1 ml. of 50% aqueous potassium hydroxide solution and boil gently for 10 minutes. Cool, wash down the sides of the beaker with 30 ml. of ethyl ether, and transfer to a clean 125-ml. separatory funnel. Wash out the beaker with two 25-ml. portions of distilled water, transferring each to the separatory funnel. Rotate the funnel, but do not shake. Break any emulsion with 5 ml. of alcohol. Withdraw the soap solution from the bottom. Wash the ether layer 3 times with 50-ml. portions of distilled water. If the last washings are not neutral to phenolphthalein, repeat until washings are neutral.

Pour the ether layer into a 50-ml. beaker and evaporate on a steam or water bath until all moisture is gone. Cool and pipet 5.0 ml. of chloroform into the beaker, swirling gently to ensure solution. Transfer the solution to a clean, dry 15 × 150 mm. test tube and place in a lightproof constant-temperature cabinet at 18° C. for 5 minutes. Proceed as directed above and determine the transmittance of each solution. From the standard curve find the concentration of cholesterol corresponding to th transmittance.

Prepare a reagent blank by placing the total solvents used fo one sample in a 250-ml. beaker and evaporating as directed abov Carry through all the steps in the procedure and read the trans mittance. Find the concentration of cholesterol correspondin to this transmittance from the standard curve.

CALCULATIONS. Grams of cholesterol in sample equal tot grams of cholesterol found in sample minus grams of cholesterol i reagent blank.

Find per cent yolk present by consulting Table IV.

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Determination of Boric Oxide in Glass

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A method has been developed for the determination of boron in glass which is more accurate and more rapid than the methods commonly used. Data obtained on synthetic mixtures and actual glass samples show that this method yields results with a mean error, signs disregarded, of 0.04% B_2O_3 with glasses containing less than 15% B_2O_3.

THE essential differences among the various methods for the determination of boric oxide in glass lie in the method of separating the interfering substances, such as iron, aluminum, or silica, from the solution containing the boric acid, which is then titrated with sodium hydroxide in the presence of mannitol. Thus, Wherry and Chapin (*5*) dissolve the sodium carbonate fusion mixture in hydrochloric acid, and add calcium carbonate in moderate exceess in order to neutralize the free hydrochloric acid and to precipitate the sesquioxides and most of the silica. In another procedure (*5*), they recommend separation of the boric acid from the solution by distillation of the methyl ester. Glaze and Finn (*1*), on the other hand, base their method on the partition of boric acid between water and ethyl ether in the presence of ethyl alcohol and a small amount of sulfuric acid.

The method described in this paper is more accurate and less time-consuming than procedures which have been in use heretofore.

EXPERIMENTAL

PROCEDURE WITH pH METER. Fuse a 500-mg. sample with 3 grams of sodium carbonate for 10 minutes. Dissolve the melt in 20 ml. of 6 *N* hydrochloric acid and adjust the pH of the solution to 5.0 to 5.5 by means of 6 *N* sodium hydroxide. Aluminum, iron, and similar elements, along with most of the silica, are precipitated at this point. Sweep out the carbon dioxide by bubbling air (purified by passing through concentrated sulfuric acid and Ascarite) through the solution at 60° ± 5° C. for 30 minutes. Filter the reaction mixture, and wash the precipitate with warm water until the volume of the filtrate is 250 ml. Cool the solution to room temperature, and adjust its pH to 6.30 by means of carbonate-free 0.05 *N* sodium hydroxide. Add 40 grams of mannitol to the solution. The pH is decreased markedly by the formation of mannitoboric acid. Now titrate to a pH of 6.30 again with 0.05 *N* sodium hydroxide. The alkali used in this titration is a measure of the boron in the sample.

In some cases, a double precipitation of the residue is necessary to recover the coprecipitated boric acid. In order to accomplish this, dissolve the precipitate in 10 ml. of 6 *N* hydrochloric acid, and repeat the same procedure, running a separate titration o the second filtrate.

PROCEDURE WITH INDICATOR. Fuse and dissolve the sampl as above, then add 20 drops of 0.04% bromocresol purple. Ad 6 *N* sodium hydroxide until the first distinct color change (yellow to dirty green) can be recognized. At this point, the pH of th solution is approximately 5.5. Then proceed as in the pH mete method. Adjust the pH to 6.30 by comparing the color of th solution with that of a phosphate comparison buffer.

TITRATION CORRECTIONS. The regular titration of boric aci with sodium hydroxide in the presence of mannitol, following th

Table I. Titration Corrections

$HBO_2.H_2O$ Found, Milliequivalents	Corrections, Milliequivalent	$HBO_2.H_2O$ Found, Milliequivalents	Corrections, Milliequivalent
0.0	0.000	1.2	0.024
0.1	0.005	1.4	0.026
0.2	0.008	1.6	0.029
0.4	0.013	1.8	0.031
0.6	0.016	2.0	0.033
0.8	0.019	2.4	0.036
1.0	0.022	2.8	0.040

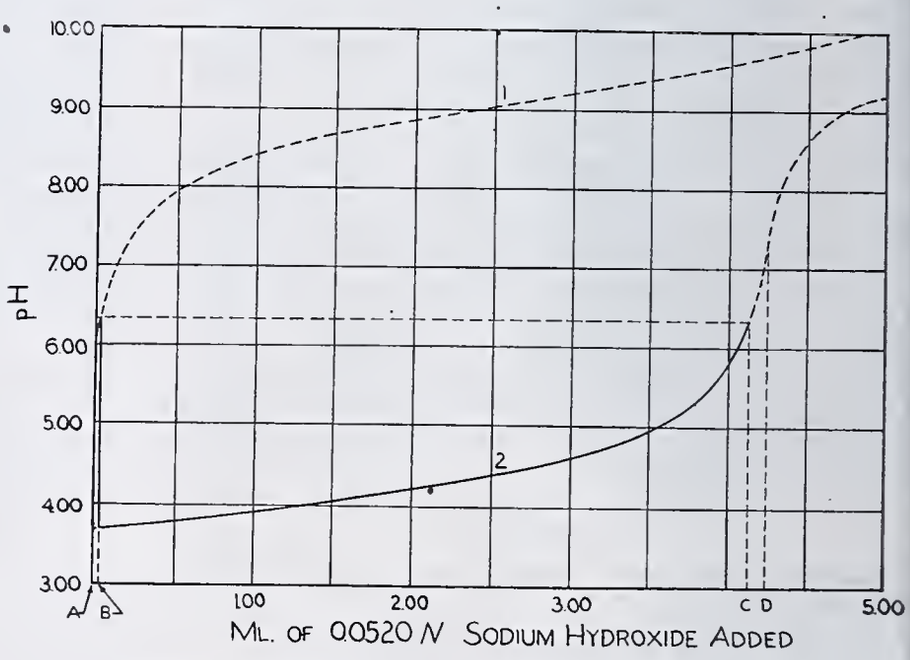

Figure 1. Potentiometric Titration Curves for Boric Acid

1. Without mannitol
2. With mannitol

directions of Hollander and Rieman (*2*), cannot be employed because of the interference of varying amounts of carbon dioxide and colloidal silica which remain in the solution. Therefore, the method of titration illustrated by the solid parts of the curves in Figure 1 is used. The first adjustment of the pH to 6.30 results in the neutralization of a small fraction of the boric acid along curve 1, for which correction *AB* must be applied. Upon the addition of mannitol, the boric acid is converted to the stronger mannitoboric acid; consequently, the pH of the solution is depressed markedly. This is illustrated by the solid vertical line connecting the two curves. The second adjustment of the pH to 6.30 follows the path of curve 2. The pH of 6.30 is reached before the equivalence point (steepest part of curve 2). Therefore, another correction corresponding to volume *CD* must be applied.

The total corrections, *AB* + *CD*, for various amounts of boric acid are given in Table I. These data were obtained by potentiometric titrations of the indicated quantities of boric acid and of mannitoboric acid under the recommended conditions and subsequent interpretation of the graphs as explained above. The correction is to be added in all cases.

The recommended conditions for the titration—i.e., an initial and final pH of 6.30 and the addition of 40 grams of mannitol—were chosen because they give minimum corrections. The recommended initial volume of 250 ml. is the smallest that can be used without evaporation if the precipitate is to be washed thoroughly.

Small amounts of carbonic acid and silica may be present in the solution during the titration without interfering because the pH is the same at the end as at the beginning. Large amounts of these substances interfere, however, by giving small slopes to the graph in the vicinity of pH = 6.30, thus making it impossible to measure accurately the sodium hydroxide required for the titration. The recommended procedure leaves only small amounts of these substances in the solution.

Table II. Compositions of Synthetic Mixtures

Constituent	Mixture Number 1	2	3	4	5	6	7[a]	8	9	10	11
B_2O_3	1.47	2.0	3.0	4.13	6.15	7.22	12.76	18.3	41.0	42.8	71.8
SiO_2	40.16	67.3	..	66.80	37.04	66.90	80.60	52.40	..	..	..
Al_2O_3	2.90	2.5	10.0	2.54	3.70	6.38	1.94	1.9	..	5.0	22.4
Fe_2O_3	...	...	..	0.17	...	0.22	0.076	...	..	..	..
TiO_2	...	...	..	...	...	...	0.027	...	..	..	..
ZrO_2	...	...	..	...	...	...	0.013	...	..	..	..
CaO	7.21	7.0	..	1.73	...	7.94	....	0.3	..	..	..
MgO	...	0.17	4.0	2.60	...	0.61	0.026	...	..	..	..
BaO	...	...	..	...	42.04	...	....	...	..	..	..
Na_2O	12.06	14.0	..	7.40	...	1.25	4.16	2.3	..	..	..
K_2O	2.00	...	12.0	1.75	...	2.40	0.16	4.3	..	..	..
Li_2O	...	...	..	...	...	...	....	...	..	..	5.8
PbO	25.50	...	..	...	1.26	...	....	...	..	52.0	..
ZnO	...	7.0	..	9.75	9.25	...	....	...	59.0	..	..
Mn_2O_3	...	0.28	..	...	...	...	....	...	..	..	..
P_2O_5	...	...	70.5	...	...	...	....	...	..	..	..
Sb_2O_3	...	...	..	1.29	...	...	....	20.4	..	..	..
As_2O_3	...	...	0.5	2.05	0.56	...	0.14	0.1	..	..	..
As_2O_5	...	...	..	...	...	...	0.085	...	..	..	..
SO_3	1.0	...	..	...	...	...	0.009	...	..	..	..
F	7.10	...	..	...	...	...	....	...	..	..	..
Cl	...	...	..	...	...	...	0.036	...	..	..	..

[a] Composition given on certificate of glass 93 of National Bureau of Standards.

Table III. Determination of Boric Oxide in Synthetic Mixtures by pH Meter

Mixture No.	No. of Determinations	Average Error, %	Mean Deviation, %
1	1	−0.03	...
2	3	+0.03	0.03
3	3	−0.03[a]	0.03
4	4	+0.06	0.03
5	6	+0.03	0.03
6	1	−0.03	...
7	4	−0.04	0.03
8	1	−0.10	...
9	2	−0.20	0.07
10	3	−0.03	0.03
11	4	−0.36	0.10

[a] Results obtained by modified method designed to remove phosphate.

Table IV. Determination of Boric Oxide in Glasses

Glass	B_2O_3 Reported, %	B_2O_3 Found, %	Difference, %	Mean Deviation, %	No. of Precipitations	Method
Bureau of Standards 128	1.52	1.52[a]	0.00	0.00	One	pH meter
Bureau of Standards 93	12.76	12.51[b]	0.25	0.01	Two	pH meter
Corning, 1	0.61	0.59	0.02	0.01	One	pH meter
Corning, 3	ca. 14.7	14.71	0.0	0.0	Two	pH meter
Armstrong 224*abc*	9.80[c]	9.91	0.11	.01	Two	pH meter
Armstrong 427*abc*	9.75[c]	9.66	0.09	0.02	Two	pH meter
Bureau of Standards, 92	0.70	0.65	0.05	0.01	One	Indicator
Bureau of Standards, 128	1.52	1.50	0.02	0.01	One	Indicator

[a] Corrected for change in loss on ignition.
[b] Dried at 500°.
[c] By method of Glaze and Finn.

RESULTS

Several synthetic mixtures, similar to some glass compositions given by Morey (*3*), were analyzed by this method (Tables II and III). Several glasses were also analyzed (Table IV).

DISCUSSION

Table III shows that the boric oxide content of glasses with less than 15% B_2O_3 can be determined by the pH meter method with a mean error of ±0.04% B_2O_3 without interference from Al_2O_3, Fe_2O_3, CaO, BaO, MgO, K_2O, Li_2O, PbO, ZnO, Mn_2O_3, Sb_2O_3, As_2O_3 (As_2O_5), TiO_2, ZrO_2, SO_3, and F.

Phosphate causes high results unless the melt is dissolved in 6 *N* nitric acid and the phosphate removed by the addition of a slight excess of silver nitrate at pH 5.5. This interference was also mentioned by Ruehle and Shock (*4*). It is caused by the activation of the phosphoric acid by mannitol.

The authors' method yielded results with a mean error of −0.04% B_2O_3 on a synthetic mixture (No. 7) identical in composition with the certified value of glass 93 of the National Bureau of Standards. The authors believe, therefore, that the true boric oxide content of this glass is not 12.76%, but nearer 12.51%.

The indicator method gives satisfactory results for glasses with low boric oxide contents. High-boron glasses will buffer the solution too much to yield distinct color changes by which the end point can be recognized.

This method is probably applicable to many samples other than glasses.

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The pH of Wines

Examination of Glass and Quinhydrone Electrode Values

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Values obtained for the pH of wines by means of the glass and quinhydrone electrodes sometimes agree, but often differ by more than 0.1 pH unit. The effects of several constituents of wine on these electrode systems have been examined and it is concluded that, whereas the glass electrode values are essentially correct, the quinhydrone electrode, when used in wines, is subject to at least two sources of error. Alcohol causes the quinhydrone electrode to register low values, while reducing substances, such as sulfites and tannin, lead to high values. The correct and near-correct values obtained with the quinhydrone electrode in some wines are due to a compensation of such errors. Neither the hydro-quinhydrone nor the quino-quinhydrone electrodes give more reliable results in wines than the regular quinhydrone electrode.

THE significance of pH values in relation to the physical, chemical, and biological changes which occur in wine has been pointed out by many investigators, notably Ventre (*17*), Genevois and Ribéreau-Gayon (*11*), Brémond (*3*), and Ribéreau-Gayon (*14, 15*). The present writer (*10*) has indicated the importance of pH in relation to bacterial spoilage of wines.

Of the various methods available for determining the pH value of wine, the quinhydrone electrode system has been one of the most widely used. Chief among the advantages claimed for this system are that it is easily and quickly assembled, simple to operate, reaches a stable potential rapidly, and can be used in wines which contain small to moderate amounts of sulfites. Because of these features it has been widely recommended for determining the pH values of wines (*3, 6, 11, 16*). Within recent years, however, improvements in the design and construction of glass electrodes and vacuum tube potentiometers have resulted in the introduction of the glass electrode system into industrial laboratories and an increasing number of glass electrode pH meters are now being used in wineries.

From time to time, discrepancies have been noticed between the results obtained by means of the glass electrode and the quinhydrone electrode for the pH values of wines. Thus Ribéreau-Gayon (*16*) found that the pH values of wines determined by means of the glass electrode were slightly (up to 0.06) higher than those registered by the quinhydrone electrode. Using tartrate buffers containing different amounts of alcohol, he obtained evidence that the deviation was due to alcohol, but did not indicate which electrode system gave the more correct values. Hooper (*12*) recorded differences between glass electrode and quinhydrone electrode values for several Australian fortified wines, which varied from 0.05 to 0.18 pH unit. Liebmann and Rosenblatt (*13*) determined the pH values of different whiskies by means of the glass and the quinhydrone electrodes and found the quinhydrone electrode values lower than the glass electrode values by about 0.37 pH unit. They calculated, from experimental data obtained by Dole (*8*), that the theoretical deviation of the glass electrode values in 45% alcohol was only about 0.03 pH unit and they therefore concluded that the glass electrode values for whisky were essentially correct and the quinhydrone electrode values in error by about −0.37 pH unit.

In view of the foregoing, it seemed desirable to make a more detailed examination of the values obtained with the glass and quinhydrone electrode systems for the pH of wines.

APPARATUS AND REAGENTS

ELECTRODE SYSTEMS. *Glass Electrode System.* The glass electrode was a Kerridge pattern recessed bulb type, having the bulb blown of Corning 015 special electrode glass. This was used with an internal quinhydrone–0.1 *N* hydrochloric acid half-cell and a saturated calomel half-cell as a reference half-cell. Between determinations the outside of the bulb of the glass electrode was rinsed with distilled water, dried lightly with filter paper, and then rinsed with a portion of the next test solution. When not in use, the glass electrode was kept filled with, and immersed in, distilled water.

Leads from the electrodes to the potentiometer were of insulated copper wire, kept as short as possible, and the electrode clamps were supported in blocks of polystyrene.

Quinhydrone Electrode System. The electrodes were made of pieces of 10 × 7 mm. platinum foil, each welded to a length of platinum wire and sealed into a glass tube. Electrical connections were made by means of binding screws at the top of the wire. The electrodes were heated to redness in an alcohol flame for a few seconds before use and at frequent intervals during each batch of determinations.

Quinhydrone freshly prepared from hydroquinone and ferric ammonium alum according to the method of Biilmann, described by Britton (*4*), was washed four times with iced distilled water and dried between filter paper at room temperature. Since the presence of traces of iron in quinhydrone prepared by Biilmann's method may be a source of error, a batch of quinhydrone prepared by mixing alcoholic solutions of quinone and hydroquinone according to the method of Valeur (described by Britton, *4*), was also used for comparison. This latter product was in a coarser state of division and was rather slower in giving stable potentials than that prepared by Biilmann's method, but otherwise no significant differences were noticed between the results obtained with the two preparations.

Bubbling Hydrogen Electrode System. The platinum electrodes were of similar construction to those described above. They were cleaned by heating in dilute (1 + 1) aqua regia and lightly platinized, so that only thin brown coatings of platinum black were obtained. Before each determination, these electrodes were connected to the negative pole of a 4-volt storage battery, electrolyzed for half a minute in 0.1 *N* sulfuric acid, then carefully washed with distilled water before being placed in the test solution. All determinations were made in duplicate, using two electrodes and electrode vessels, and when discrepancies occurred, the electrodes were cleaned and replatinized.

The electrode vessels were a modification (*2*) of the bubbling hydrogen electrode vessel described by Best (*1*).

REFERENCE HALF-CELL. A saturated calomel half-cell was used in conjunction with each of the above electrode systems.

LIQUID JUNCTIONS. Liquid junctions between the calomel cell and the test solutions were formed by means of a side tube which was kept filled with a saturated solution of potassium chloride from a reservoir, and which was closed with a ground-glass cap. Before each determination the lower end of the tube was washed with distilled water and dried with filter paper. The junction was then renewed by loosening the cap momentarily to allow a few drops of the potassium chloride solution to escape and the outside of the cap was lightly wiped with filter paper.

POTENTIOMETER. pH measurements were made with a vacuum tube potentiometer calibrated to give readings directly in pH units, and connected to a 12-volt storage battery as a source of current. The calibration of the instrument was checked by using it with a quinhydrone electrode, to measure the pH values of the buffers listed below, and comparing these values with those obtained for the same buffers and with the same electrode, by means of a Cambridge portable potentiometer. The values obtained with the two instruments did not differ by more than 0.02 pH unit in any of these solutions.

BUFFER SOLUTIONS. The reagents used in the preparation of buffer solutions were all of analytical reagent grade.

For standardizing and checking the electrode systems the following solutions were used:

0.05 *M* potassium hydrogen phthalate	pH 4.01 (3.97)
Sorensen's phosphate buffer	pH 6.26 (6.24)
McIlvaine's citrate-phosphate buffer	pH 4.63 (4.6)

The pH values in parentheses are those given by Clark (*5*) for these solutions, but in these investigations the value of 4.01 for

Table I. pH Values of Wines

Type	No.	Alcohol % by Volume	pH Values: Glass electrode	pH Values: Quinhydrone electrode	pH Values: Deviation of quinhydrone from glass
Sweet red	1	20	3.74	3.50	−0.24
	2	20	3.72	3.52	−0.20
	3	20	3.71	3.56	−0.15
	4	20	3.84	3.69	−0.15
	5	20	3.66	3.52	−0.14
	6	20	3.45	3.29	−0.16
	7	20	3.69	3.56	−0.13
	8	20	3.73	3.62	−0.11
	9	19	3.64	3.52	−0.12
	10	19	3.64	3.52	−0.12
Sweet white (spoiled)	11	19	4.29	4.16	−0.13
Muscat	12	20	3.94	3.81	−0.13
Dry white	13	14	3.21	3.18	−0.03
Dry white (carrying sherry flor)	14	13	3.32	3.30	−0.02
	15	15	3.31	3.24	−0.07
Dry white (sherry)	16	17.5	3.40	3.30	−0.10
Dry white	17	14.5	3.32	3.26	−0.06
	18	15	3.59	3.56	−0.03
	19	13.5	3.73	3.70	−0.03
	20	14.0	3.80	3.77	−0.03
	21	11.5	2.95	2.93	−0.02
	22	10.0	3.22	3.22	0.00
Dry red	23	11.5	3.48	3.58	+0.10
	24	11.5	3.53	3.65	+0.12
White grape juice (pasteurized)	25	..	3.33	3.34	+0.01
	26	..	3.42	3.41	−0.01

the pH of 0.05 *M* phthalate was adopted as the primary standard in accordance with the recommendation of Dole (*7*). The pH values of the Sorensen and McIlvaine buffers were then found to be 6.26 and 4.63, respectively, by means of the glass electrode.

The effect of ethyl alcohol on pH determinations was studied with phthalate, tartrate, and citrate buffers, the last two being used instead of the phosphate buffers previously mentioned, as it was considered that they would provide conditions more comparable with those in wine. These solutions were made up in the following concentrations:

0.1 *M* potassium hydrogen phthalate
0.1 *M* tartrate solution. 15.0 grams of tartaric acid and 80 ml. of 1.0 *N* sodium hydroxide per liter
0.066 *M* citrate solution. 14.0 grams of citric acid and 68 ml. of 1.0 *N* sodium hydroxide per liter

Hundred-milliliter quantities of these solutions were diluted with 100-ml. quantities of appropriate alcohol-water mixtures before use, so that the final concentrations were 0.05 *M* phthalate, 0.05 *M* tartrate, and 0.033 *M* citrate and the pH values of the diluted aqueous solutions were found by means of the glass electrode to be 4.01, 3.33, and 3.87, respectively.

EXPERIMENTAL

All determinations were carried out at room temperature, which was 15° ± 2° C. during the course of the investigations, but the variation never exceeded 0.5° C. during any one batch of determinations.

Standardizing and Checking Electrode Systems and Potentiometer. Before each batch of determinations with the glass or quinhydrone electrodes, the potentiometer was adjusted to give a reading of 4.01 for the pH of the 0.05 *M* phthalate buffer. The electrode system and potentiometer were then further checked against the phosphate and the citrate-phosphate buffers before other measurements were made. The check against these three buffers was repeated at the end of each batch of determinations and if the pH values recorded in the second check differed from those first obtained by more than ±0.02, the whole batch of determinations was repeated with fresh samples. With electrode systems other than the glass and quinhydrone, the potentiometer was standardized as indicated in Tables II and V.

The pH values obtained for the phosphate and citrate-phosphate buffers on different days provide an indication of the reproducibility of results. The standard deviations calculated from these results did not exceed ±0.02 for either the glass or the quinhydrone electrode. The standard deviations of the values obtained for wines were not determined statistically, but all determinations were made in duplicate and the mean values taken. When, as rarely happened, the values obtained for duplicate samples differed by more than 0.02, the determination was repeated with fresh samples. This procedure could not be followed with the quinhydrone values recorded in Table IV, however, for in those samples which showed appreciable drift in potential, agreement between duplicates was usually unsatisfactory. Except in this special case, however, it is considered that under the experimental conditions differences exceeding ±0.03 pH unit are significant.

Determination of pH Values of Wines by Means of Glass and Quinhydrone Electrodes. The pH values of several wines as determined by the glass and the quinhydrone electrodes are shown in Table I. As can be seen, the glass electrode values are nearly all higher than the quinhydrone electrode values, but the differences between them vary considerably from one wine to another.

Effect of Ethyl Alcohol on Glass and Quinhydrone Electrodes. In view of the results obtained by Ribéreau-Gayon (*16*) and by Liebmann and Rosenblatt (*13*) concerning the effects of ethyl alcohol, it seemed desirable to determine the extent to which differences such as those recorded in Table I were due to the alcohol present in the wine. Accordingly, the pH values of phthalate, tartrate, and citrate buffers containing different concentrations of ethyl alcohol were determined by means of the glass and the quinhydrone electrodes (Table II).

Duboux and Tsamados (*9*) have shown that the dissociation of organic acids is depressed by the presence of ethyl alcohol and so, as would be expected, the pH values found for the buffer solutions in Table II increased with increasing alcohol content. Although it seemed likely from the data of Liebmann and Rosenblatt (*13*) that the glass electrode values would be correct, it was considered desirable to check these values with the bubbling hydrogen electrode as a standard. The hydrogen electrode values for the tartrate and citrate solutions are shown in column 3 of Table II, and as can be seen, they agree well with the glass electrode values.

A comparison of the results presented in Tables I and II indicates that the deviations of the quinhydrone from the glass electrode values in wines are often smaller than would be expected from a consideration of the amounts of alcohol present. It seems likely, therefore, that in some wines the error in quinhydrone electrode values due to alcohol is in part offset by some other factors which cause an apparent increase in pH values. In order to obtain more information on this point several wines were treated as follows: 200 ml. of wine were evaporated to about 40 ml. under reduced pressure at 60° C. to remove alcohol. Half of each de-

Table II. pH Values of Buffers Containing Ethyl Alcohol

Buffer	Alcohol Content % by Volume	pH Values: Hydrogen electrode	pH Values: Glass electrode	pH Values: Deviation of glass from hydrogen	pH Values: Quinhydrone electrode	pH Values: Deviation of quinhydrone from glass
0.05 *M* phthalate	None	...	4.01[a]	....	4.01[a]	....
	10	...	4.21	....	4.14	−0.07
	20	...	4.44	....	4.30	−0.14
Tartrate	None	3.33[a]	3.33[a]	....	3.33[a]	....
	5	3.40	3.40	0.00	3.36	−0.04
	10	3.47	3.47	0.00	3.40	−0.07
	15	3.55	3.54	−0.01	3.43	−0.11
	20	3.64	3.63	−0.01	3.48	−0.15
	25	3.75	3.73	−0.02	3.52	−0.21
Citrate	None	3.87[a]	3.87[a]	....	3.87[a]	....
	5	3.94	3.94	0.00	3.91	−0.03
	10	4.01	4.01	0.00	3.95	−0.06
	15	4.08	4.08	0.00	3.99	−0.09
	20	4.16	4.15	−0.01	4.03	−0.12

[a] In each case potentiometer and electrode system was standardized to give correct value for aqueous buffer solution.

Table III. Effect of Alcohol on pH Values of Wines as Measured by Glass and Quinhydrone Electrodes

Wine No.	7	9	11	12	13	14
Original wines						
Alcohol content, volume %	20	20	19	20	14	13
pH by glass electrode	3.69	3.64	4.29	3.94	3.21	3.32
pH by quinhydrone	3.56	3.52	4.16	3.81	3.18	3.30
Quinhydrone deviation	−0.13	−0.12	−0.13	−0.13	−0.03	−0.02
Dealcoholized wines						
pH by glass electrode	3.37	3.36	4.09	3.62	3.07	3.17
pH by quinhydrone	3.40	3.41	4.07	3.64	3.15	3.23
Quinhydrone deviation	+0.03	+0.05	−0.02	+0.02	+0.08	+0.06
Reconstituted wines						
pH by glass electrode	3.67	3.65	4.36	3.93	3.23	3.32
pH by quinhydrone	3.57	3.56	4.18	3.80	3.21	3.29
Quinhydrone deviation	−0.10	−0.09	−0.18	−0.13	−0.02	−0.03

alcoholized wine was then made up to 100 ml. with distilled water, while the other half was made up to 100 ml. with distilled water and ethyl alcohol, so that the final alcohol content of the treated wine was the same as that of the original wine. The pH values of the treated wines were determined by the glass and the quinhydrone electrodes. These values together with those of the original wines and the quinhydrone electrode deviation in each case are shown in Table III.

These results show that:

The pH values and the quinhydrone electrode deviations for the reconstituted wines agree reasonably well with those found for the original wines except for wine 11. This wine had undergone bacterial spoilage and had a high volatile acidity. The loss of a portion of the volatile acid during evaporation probably accounts for the higher pH value of the reconstituted wine.

The quinhydrone electrode values of several of the dealcoholized wines are slightly higher than the glass electrode values. This is particularly noticeable in wines 13 and 14, the quinhydrone electrode values of which were only slightly below the glass electrode values before treatment. Removal of the alcohol from these wines thus reveals the presence of factors which cause a positive error in the quinhydrone electrode values, an error which in the untreated wine partially or wholly offsets the negative error caused by alcohol.

It is well known that the quinhydrone electrode is subject to errors in the presence of oxidizing or reducing substances which are sufficiently active to change the ratio of quinone to hydroquinone in solution. It seemed likely, therefore, that the high quinhydrone electrode values obtained in some of the dealcoholized wines were due to reduction of quinone to hydroquinone by some constituents of the wines.

Effect of Tannin, Sulfite, and Acetaldehyde on Glass and Quinhydrone Electrodes. In order to study the influence on the quinhydrone electrode of some of the reducing substances which occur in wine, tannin, sulfite, and acetaldehyde were added to the aqueous tartrate solution and to a dry white wine in amounts which are comparable with those occurring in wines. The tannin used was Merck's analytical reagent tannic acid, the sulfite was added as potassium metabisulfite, and the acetaldehyde was added as a 10% solution of acetaldehyde. The pH values of the treated wines and buffer solutions were determined with the glass and the quinhydrone electrodes and the results are shown in Table IV.

None of the additions had any significant effect on the glass electrode values, but the tannin and the sulfite affected the quinhydrone electrode values, appreciably both in the tartrate buffer and in the wine, although the effects were less marked in the wine. Moreover, the sulfite caused the quinhydrone electrode potential to drift both in the tartrate buffer and in the wine, while the tannin caused a drift in the tartrate buffer but not in the wine. As can be seen from the table, the drift due to sulfite was different from that due to tannin. The addition of acetaldehyde did not affect the quinhydrone electrode values appreciably either in the tartrate buffer or in the wine.

The Hydro-quinhydrone and Quino-quinhydrone Electrodes. According to Clark (*5*) the effects of side reactions in disturbing the ratio of quinone to hydroquinone in the quinhydrone electrode system can sometimes be eliminated by saturating the test solution with quinone and quinhydrone or with hydroquinone and quinhydrone. It was thought that such systems might give better results in wines than the ordinary quinhydrone electrode system. Values obtained in wines, however, were not found more reliable than those obtained with the regular quinhydrone system.

The figures presented in Table V show the results obtained in the presence of varying amounts of alcohol in the tartrate buffer with the glass, quinhydrone, quino-quinhydrone, and hydro-quinhydrone electrodes. The results obtained with the glass, quinhydrone and quino-quinhydrone electrodes in several wines are also shown. In order to simplify comparisons, the results obtained with the quinhydrone, quino-quinhydrone, and hydro-quinhydrone electrodes are shown as deviations from the glass electrode values.

Table IV. Effect of Tannin, Sulfite, and Acetaldehyde on pH Values

Solution	Additions	pH Values: Glass electrode	Quinhydrone Electrode: 0.5 min.	5 min.	30 min.
Tartrate buffer (0% alcohol)	None	3.33	3.33	3.33	3.33
	Tannin 1 gram per liter	3.32	3.35	..	3.37
	Tannin 2 grams per liter	3.32	3.36	..	3.40
	$K_2S_2O_5$ 100 p.p.m.	3.33	3.43	3.46	3.40
	$K_2S_2O_5$ 200 p.p.m.	3.32	3.48	3.52	3.46
	$K_2S_2O_5$ 400 p.p.m.	3.32	3.73	3.97	3.61
	CH_3CHO 200 p.p.m.	3.33	3.35	..	3.34
	CH_3CHO 400 p.p.m.	3.33	3.35	..	3.34
Dry white wine (14.5% alcohol)	None	3.32	3.26	..	3.26
	Tannin 2 grams per liter	3.32	3.31	..	3.31
	$K_2S_2O_5$ 200 p.p.m.	3.32	3.27	3.30	3.28
	$K_2S_2O_5$ 400 p.p.m.	3.32	3.51	3.57	3.36
	CH_3CHO 400 p.p.m.	3.32	3.26	..	3.25

As can be seen from the table the error due to alcohol was even greater with the hydro-quinhydrone than with the regular quinhydrone system. On the other hand, the quino-quinhydrone system, although giving pH values showing reasonably good agreement with those obtained by means of the glass electrode in buffers containing alcohol, appeared to be greatly affected by those constituents of wine which cause the quinhydrone electrode to give high values. Thus in wines 12 and 16 for which the quinhydrone electrode values were considerably lower than the glass electrode values, the quino-quinhydrone electrode values agreed well with those of the glass electrode. In wines 13, 14, and 18, for which the quinhydrone electrode values were only slightly lower than the glass electrode values, however, the quino-quinhydrone electrode gave values appreciably higher than those obtained with the glass electrode.

DISCUSSION

The pH values of wines as determined by means of the glass electrode are essentially correct, for the glass electrode is not affected by the concentrations of ethyl alcohol that occur in wines nor by oxidation-reduction systems. On the other hand the quinhydrone electrode, when used in wines, is subject to at least two sources of error.

Table V. Comparison of pH Values

Solution	Alcohol Content % by Vol.	pH, Glass Electrode	pH Values with Different Electrodes Expressed as Deviations from Glass Electrode Values: Quinhydrone	Hydroquinhydrone	Quinoquinhydrone
Tartrate buffer	None	3.33	0.00[a]	0.00[a]	0.00[a]
	5	3.40	−0.04	−0.10	+0.02
	10	3.47	−0.07	−0.12	+0.02
	15	3.54	−0.11	−0.17	+0.03
	20	3.63	−0.15	−0.17	+0.02
	25	3.73	−0.21	−0.19	0.00
Wine 11	19	4.29	−0.13	.....	+0.06
12	20	3.94	−0.13	.....	−0.02
13	14	3.21	−0.03	.....	+0.07
14	13	3.32	−0.02	.....	+0.06
15	15	3.31	−0.07	.....	+0.02
16	17.5	3.40	−0.10	.....	+0.01
17	14.5	3.32	−0.06	.....	+0.01
18	15	3.59	−0.03	.....	+0.20

[a] Potentiometer set to give value of pH 3.33 for aqueous tartrate buffer with each electrode system.

Alcohol causes the quinhydrone electrode to register pH values which are too low, possibly because of the greater increase in the solubility of quinone in comparison with that of hydroquinone as the solvent is changed from water to alcohol. Within the range of alcohol concentrations studied in buffer solutions, the deviation of the quinhydrone electrode values from those obtained with the glass electrode appears to bear a linear relationship to the alcohol content of the solution.

Reducing substances such as tannin, sulfites, and probably the coloring matter of red wines cause the quinhydrone electrode to register pH values which are too high, presumably because of the reduction of quinone to hydroquinone.

Because these two sources of error operate in opposite directions, the quinhydrone electrode values for many wines, particularly those of low to moderate alcohol content, are reasonably good approximations of the glass electrode values. In other wines, however, particularly fortified wines, the quinhydrone electrode may give values which are more than 0.15 pH unit too low without showing any appreciable drift in potential. In some light wines, particularly those with a high tannin content and those which have been heavily sulfited, the quinhydrone electrode values may actually be too high. In such wines, however, the drift in potential would probably warn the operator that something was wrong.

Liebmann and Rosenblatt (*13*) concluded that since the only appreciable error to which the quinhydrone electrode was subject in whisky was that due to alcohol, the pH of whisky could be determined equally accurately by either the glass or the quinhydrone electrode, provided that a correction was applied to the quinhydrone electrode values to compensate for the alcohol error. No such simple correction can be applied to the quinhydrone electrode values for wines, however, for in wines the errors are not caused by a single factor and are not constant in amount. For this reason the glass electrode system appears to provide the most satisfactory means of determining the pH values of wines accurately.

SUMMARY

The pH values of wines and buffer solutions containing alcohol have been determined by the glass, quinhydrone, quino-quinhydrone, and hydro-quinhydrone electrodes and the alcohol-containing buffer solutions have been checked with the bubbling hydrogen electrode. Values obtained by the glass electrode were found to be essentially correct.

The effects of the presence of tannin, sulfites, acetaldehyde, and alcohol at various concentrations on the readings of the glass and the quinhydrone electrodes were examined. None of these influenced the glass electrode significantly, but the presence of alcohol causes the quinhydrone electrode to register values which are too low, while tannin and sulfites cause errors in the opposite direction.

In wines the pH values recorded by the quinhydrone electrode are usually lower than the glass electrode values. It was concluded that both types of errors occur in wines, a positive error being caused by the presence of tannin and sulfites and a negative one by alcohol. In a number of samples, correct and near-correct values given by the quinhydrone electrode were shown to be due to a compensation of errors.

Neither the quino-quinhydrone nor the hydro-quinhydrone electrodes give more reliable results in wines than the regular quinhydrone electrode.

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(15) Ribéreau-Gayon, J., *Rev. vit.*, 83, 133, 155, 171, 181 (1935).
(16) *Ibid.*, 88, 411–15, 434–40 (1938).
(17) Ventre, J., *Ann. agr. Montpellier*, n.s. 18, 89–236 (1925).

Corrections

In the article on "Iodometric Method for the Assay of Penicillin Preparations" [Alicino, J. F., IND. ENG. CHEM., ANAL. ED., 18, 619 (1946)] errors were made in stating units.

Page 619, second paragraph under Assay of Unknowns, the first line should read: 800 to 1000 units per mg.

Page 620, Table I, the headings of the second and third columns should read: Units per mg. Table II, the heading over the last four columns should be: Units per cc. The second line under Example, below the tables, should read: bioassay 800 units per mg.

In the article on "Determination of Cuprous Chloride" [IND. ENG. CHEM., ANAL. ED., 18, 136 (1946)] under the heading Recommendations on page 137, the second sentence under Ceric Ammonium Sulfate should read: Add 250 ml. of water and 1 drop of ferrous-phenanthroline indicator solution and titrate with 0.1 *N* ceric ammonium sulfate solution (made up in 0.5 *M* sulfuric acid solution).

LEWIS F. HATCH

An omission was made in the article on "Improved Apparatus for Karl Fischer Water Determination" [IND. ENG. CHEM., ANAL. ED., 18, 726 (1946)], in failing to give credit for use of the cathode ray magic eye tube in a titrimeter. The fundamental principles involved in this application were first described by G. F. Smith and V. R. Sullivan in "Electron Beam Sectrometer", G. Frederick Smith Chemical Co., in 1936.

RICHARD KIESELBACH

In the article on "Viscosities of Pure Hydrocarbons" [IND. ENG. CHEM., ANAL. ED., 18, 611 (1946)] an error occurs in Table I. The density of *n*-octane at 20° C. is 0.702 gram per cc.

J. M. GEIST

Iodometric Microtitration for Mustard Gas

V. EVERETT KINSEY AND W. MORTON GRANT
Howe Laboratory of Ophthalmology, Harvard Medical School, Boston, Mass.

Mustard gas in amounts varying from less than 0.5 to 200 micrograms may be determined accurately by iodometric titration from the reaction of dichloramine T and mustard in organic solvents. Under the conditions outlined this reaction differentiates mustard from derivatives of mustard and is unaffected by substances which can be extracted, with the solvents used, from the biological materials tested (blood and cornea). A modification of this method, utilizing the quantitative catalyzing action of the sulfide on the reduction of dichloramine T in the presence of cyclohexanol, increases the sensitivity twentyfold in the range of 0.2 to 5 micrograms.

THE available methods for determining mustard gas (β,β'-dichloroethyl sulfide) are chiefly nephelometric and have so many other limitations that it has not been possible heretofore to do quantitative work, particularly on residual mustard in biological material. For this purpose, a simple quantitative analytical method has been developed.

The reaction described takes place in organic solvents, thereby eliminating competing hydrolysis which occurs in aqueous solution. It has a high degree of specificity for mustard compared with many mustard derivatives tested, especially when these are extracted from aqueous solutions. The test appears to be sufficiently sensitive to estimate less than 0.5 microgram of mustard with reasonable accuracy.

THE REACTION

The method is based on an oxidation, advantage being taken of the well-known reaction of dichloramine T with mustard. The amount of chlorine available from dichloramine T after a part is used in chlorinating the mustard is determined by adding potassium iodide and acetic acid and titrating the amount of iodine liberated with sodium thiosulfate. The end point is sufficiently sharp to make starch unnecessary. The difference between the amount of sodium thiosulfate required with and without mustard gives a measure of the amount of mustard in the sample. The equation for this reaction cannot be given, since the extent of the reaction of mustard varies with the concentration of dichloramine T and the solvent used to dissolve the mustard. Under the conditions in which the reaction is carried out it would appear that about 10 equivalents of chlorine are utilized per molecule of mustard.

THE SOLVENTS

In order to avoid extra blanks and possible inaccuracies resulting from side reactions of the dichloramine T with the solvents used as carriers for the mustard and the dichloramine T, the stability of dichloramine T in various solvents was determined by iodometrically measuring the amount of chlorine available at various intervals after preparation of the solutions (Table I). The solutions were kept at room temperature and not exposed to direct sunlight.

Table I. Stability of Dichloramine T

Solvent	0.01 *M* $Na_2S_2O_3$ per Ml. of 0.2% DCT Solution Titrated: Immediately	0.25 hour	1 hour	4 hours
	Ml.	*Ml.*	*Ml.*	*Ml.*
Purified kerosene (commonly known as insecticide solvent)	3.34	3.33	3.33	3.33
CCl_4, technical	3.34	3.31	3.31	3.32
Cyclohexane (practical Eastman)	3.39	3.38	3.38	3.37
Benzene, C.P.	3.32	3.30	3.28	3.27
$CHCl_3$, U.S.P.	3.33	3.33	3.34	3.33
Xylene, C.P.	3.05	3.01	2.80	2.21
Benzine, C.P.	3.34	3.21	3.18	3.05

Table II. Efficiency of Solvents

(Ml. of 0.01 *M* sodium thiosulfate for 100 micrograms of mustard)

Mustard Solvent	Dichloramine T Solvents: Purified kerosene	CCl_4	Benzene	Cyclohexane
Purified kerosene	0.58	0.73	...	0.94
CCl_4	0.80	0.59	...	1.00
Benzene	...	0.58	0.55	0.68
Cyclohexane Practical Eastman Kodak Co.	1.05	1.03	0.69	1.00
Xylene	...	0.46	...	...

From the table it may be seen that an appreciable loss of chlorinating power occurs only in xylene and benzine. Accordingly, dichloramine T was dissolved (0.2%) in carbon tetrachloride, and mustard in concentrations of 50 and 150 micrograms per ml. was dissolved in purified kerosene or in cyclohexane; 1 ml. of the chlorinating solution was added to 1 ml. of the mustard solution, and samples were withdrawn periodically over a 30-minute period for titration. Figure 1 shows that the reaction is essentially complete after 20 minutes. As a result of these experiments the reaction between dichloramine T and mustard was allowed to continue for 20 minutes in all experiments reported below.

Since the amount of mustard is represented by a difference in the amount of sodium thiosulfate required to reduce the iodine liberated by the dichloramine T in the blank and in the sample, it is obviously desirable to keep the amount of dichloramine T present to a minimum. To establish this minimum quantity, the concentration of dichloramine T was next varied from 0.10 to 0.20% and the amount of sodium thiosulfate required per microgram of mustard was determined. It was found that the

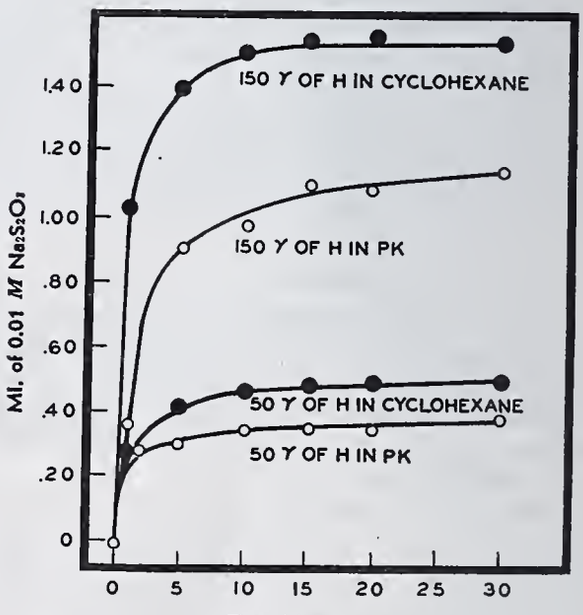

Figure 1. Rate of Reduction of Dichloramine T in Cyclohexane or Purified Kerosene Solution

H, mustard gas. PK, purified kerosene

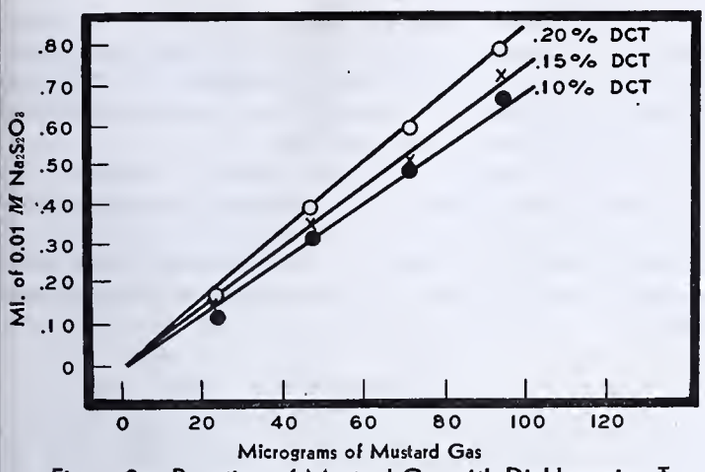

Figure 2. Reaction of Mustard Gas with Dichloramine T

DCT, dichloramine T

degree of chlorination of the mustard increased slightly over this range of concentration of dichloramine T. The results of these experiments are shown in Figure 2.

In order to find which solvent gives the best results—i.e., in which the greatest reaction occurs between the mustard and the dichloramine T—mustard (100 micrograms per ml.) and dichloramine (0.2%) were separately dissolved in different solvents and allowed to react. Table II shows the number of milliliters of 0.01 *M* sodium thiosulfate equivalent to 100 micrograms of mustard in each combination of solvents used.

The extent of the reaction is seen to be dependent upon the nature of the solvents employed. In every case the presence of cyclohexane appeared to accentuate the reaction. Because of solubility properties, carbon tetrachloride was selected as the best solvent for the dichloramine T and the mustard was dissolved in either cyclohexane or purified kerosene, for comparative purposes.

Analyses made using another lot of cyclohexane (practical, Eastman) gave somewhat different results from those shown in

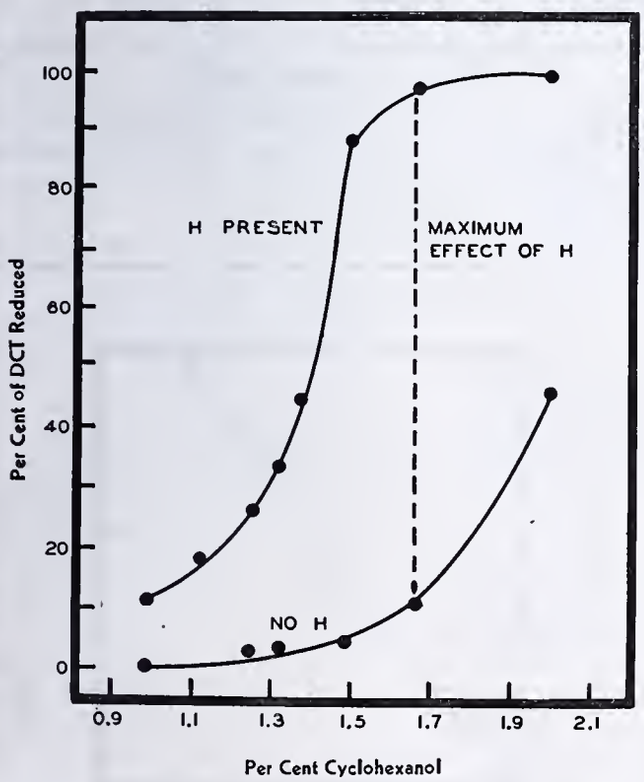

Figure 3. Proportion of Dichloramine T Reduced in 20 Minutes at 26° C. in Cyclohexane or Purified Kerosene Solutions of Varying Content of Cyclohexanol

Alone and with 6.3 micrograms of bis-β-chloroethyl sulfide, H, per ml. present

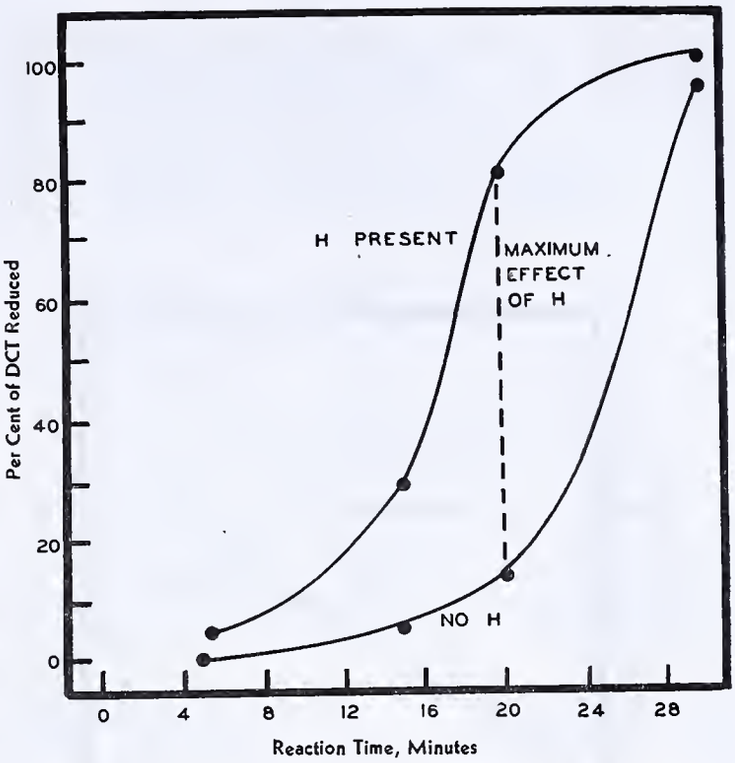

Figure 4. Proportion of Dichloramine T Reduced with Varying Time of Reaction in 1.67% Cyclohexanol Solution at 27° C.

With and without 3.33 micrograms of bis-β-chloroethyl sulfide, H, per ml. present

Table II. This suggests the advisability of running standard titration curves on each new lot of solvents or using C.P. grade cyclohexane; the latter gave a reproducible equivalent of 0.67 ml. of 0.01 *M* sodium thiosulfate per 100 micrograms of mustard and in addition had a negligible temperature coefficient.

As a result of a search for an impurity in practical cyclohexane which presumably was responsible for the difference in sensitivity, it was found that the presence of cyclohexanol could produce a manifold increase in the sensitivity of the test. While the amount of the oxidizing agent reduced per unit of the sulfide could be increased about twentyfold, the effect was found to be present only over an extremely narrow concentration range of cyclohexanol in pure cyclohexane or purified kerosene.

The relationship of the extent of reduction of dichloramine T in the presence of mustard to the concentration of cyclohexanol and the time of reaction was investigated. The amount of unreduced dichloramine T in the reaction mixtures was measured by iodometric titration. The proportion of a 0.1% solution of dichloramine T reduced in 20 minutes at 26° by 6.3 micrograms of mustard per ml. with varying concentrations of cyclohexanol present is shown in the upper curve of Figure 3. The lower curve shows the amount of dichloramine T reduced when no mustard is present. It is obvious that for the concentrations of cyclohexanol shown, the presence of mustard increases the reaction. The greatest difference between the amount of dichloramine T used by the blank and the amount used for the given amount of mustard is seen to occur at approximately 1.67% cyclohexanol in the reaction mixture.

The time of reaction of dichloramine T with mustard and cyclohexanol appears to be as critical as the concentration. Figure 4 shows that for 1.67% cyclohexanol the difference between the mustard-sensitized reduction of a 0.067% solution of dichloramine T (upper curve) and the reduction of a control solution without mustard is a maximum at 20 minutes.

PROCEDURE I

Analyses of known quantities of mustard in cyclohexane or purified kerosene were next carried out using the following technique:

One milliliter of mustard solution was allowed to react for 20 minutes with 1 ml. of 0.2% dichloramine T dissolved in carbon tetrachloride at 27° C. Four drops of saturated potassium iodide solution and 4 drops of glacial acetic acid were then added, the mixture was shaken, and 0.01 *M* sodium thiosulfate was added until no color persisted. Vigorous shaking is required as the end point of the titration is reached, and the whole procedure should be carried out away from direct sunlight. Best results are obtained by maintaining uniform temperature conditions for the mustard–dichloramine T reaction.

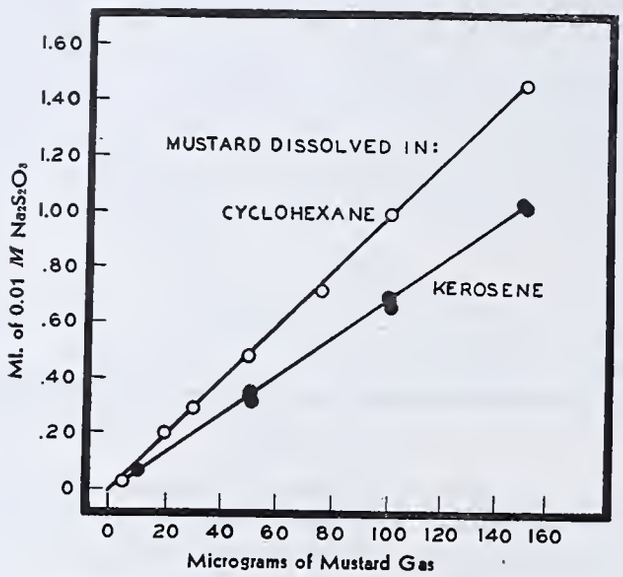

Figure 5. Results of Analyses of Mustard Gas

Concentration 0 to 160 micrograms

Figure 5 shows the results of mustard analyses performed over a range of 0 to 160 micrograms. The top and bottom lines of the figure refer to the results obtained when the mustard was dissolved in cyclohexane or purified kerosene, respectively. All the analyses in which the mustard was dissolved in purified kerosene were run separately by three different people.

Figure 6 shows the analytical results in the range of 0.5 to 4.5 micrograms. The latter quantities of mustard were contained in 0.1 ml. of purified kerosene.

For these analyses the following quantities of reagents were employed: 1 ml. of 0.2% of dichloramine T in carbon tetrachloride, 1 drop of potassium iodide, and 1 drop of glacial acetic acid. The titration was carried out with 0.02 *M* sodium thiosulfate which was contained in a micropipet.

It is clear that the usefulness of an analytical method for determining mustard in the presence of biological materials depends to a large extent upon whether derivatives of mustard or substances extracted from the biological specimens interfere with the test. Accordingly, a series of compounds closely related to mustard was prepared and dissolved in purified kerosene either to saturation, or 1000 micrograms per ml. Analyses were then made of these solutions using the technique previously outlined for determining mustard; the equivalent amount of sodium thiosulfate required is shown in Table III in comparison with that for 150 micrograms of mustard.

Table III. Interference of Derivatives

Derivatives Tested	Concentration Used in Purified Kerosene, γ/ml.	Ml. of 0.01 *M* $Na_2S_2O_3$ per Ml. of Solution: Original solution	After extraction with H_2O
$(ClCH_2CH_2)_2S$	150	1.03	1.02
$(ClCH_2CH_2)_2SO$	Saturated solution	0.35	Blank
$(ClCH_2CH_2)_2SO_2$	1000	Blank	Blank
$(HOCH_2CH_2)_2S$	Saturated solution	0.15	Blank
$(HOCH_2CH_2)_2SO$	Saturated solution	0.35	Blank
$(HOCH_2CH_2)_2SO_2$	1000	Blank	Blank

It is evident that the amount of interference of the derivatives tested, even in organic solution, is relatively slight and that no interference occurs following water extraction, no doubt because the partition coefficient of the derivatives in water and purified kerosene greatly favors the water phase.

Cornea and rabbit blood were next shaken with equal quantities of several organic solvents and the solvent was tested for the presence of titratable substances.

Table IV shows that so long as purified kerosene, cyclohexane, or xylene is used for carrying out the extraction, no interference results.

PARTITION EFFECT

In order to test whether the partition of mustard between water or blood and purified kerosene and cyclohexane sufficiently favors the organic solvent to function properly as an extractant, equal quantities of these solvents containing mustard were shaken with water and blood for 15 seconds and the loss of mustard was measured.

From Table V it may be seen that most of the mustard remains with the purified kerosene or cyclohexane when water is used to extract the mustard. When blood is used to make the extraction, cyclohexane retains most of the mustard.

From these studies of possible interfering substances and partition coefficients it is concluded that the method described may be used for making mustard analyses in biological materials.

This test carried out in the manner prescribed determines β,β′-dichloroethyl sulfide and not the first hydrolysis product of mustard, β-hydroxyethyl-β′-chloroethyl sulfide. The latter,

Table IV. Biological Material Blanks

Solvent Used	Material Extracted	Ml. of 0.01 *M* $Na_2S_2O_3$ per Ml. of Solution
Purified kerosene	Cornea	Blank
Purified kerosene	Blood	Blank
Cyclohexane	Blood	Blank
Xylene	Blood	Blank
Benzene	Blood	0.08
CCl_4	Blood	0.03

Table V. Partition Effect

Original Mustard Solution	Per Cent of Mustard Remaining after Extraction: With H_2O	With blood
In purified kerosene, 200 γ per ml.	98	88
In cyclohexane, 125 γ per ml.	99	97

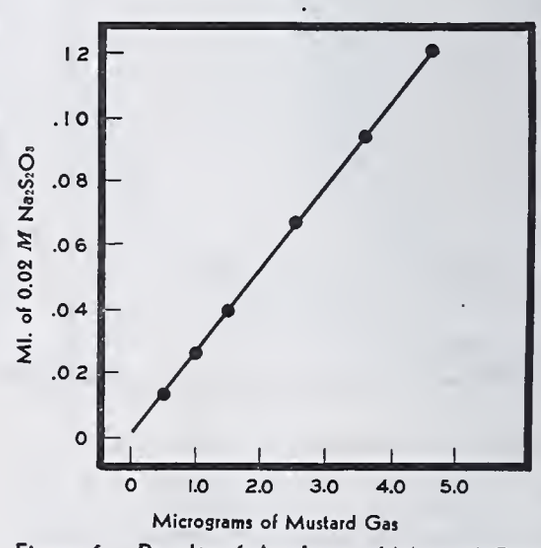

Figure 6. Results of Analyses of Mustard Gas

Concentration 0 to 5 micrograms

t was ascertained from the pure compound, is highly water-soluble and remains in the water phase when purified kerosene is used to extract the mustard; and only a trace of the hydrolysis product is extracted by cyclohexane. Sulfonium salts similarly remain in the water phase and are not determined by this method. (The isolation of β-hydroxyethyl-β′-chloroethyl sulfide and its subsequent synthesis will be described in the *Journal of the American Chemical Society.*)

The reagent with the most desirable physical and solvent properties for performing the extractions was found to consist of a mixture of 80% cyclohexane and 20% purified kerosene. Maximum and essentially complete extraction of mustard was obtained from water by vigorous shaking with an equal volume of this mixture for 20 seconds, and from rabbit blood by 2 minutes' shaking. Application of this method to a study of hydrolysis rates of mustard in water, salt solutions, and blood will be discussed in a subsequent publication in the *Journal of Biological Chemistry.*

PROCEDURE II

For applications necessitating the greatest sensitivity in the range of 0.2 to 5 micrograms of mustard gas the following procedure, which takes advantage of the sensitizing action of cyclohexanol, may be utilized: One milliliter of cyclohexane (Eastman Kodak Co., pure) containing 0 to 5 micrograms of mustard is added to 1 ml. of a 5.00% solution (by volume) of cyclohexanol in purified kerosene (Insecti-Sol insecticide solvent) or pure cyclohexane. The mixture, in a test tube, is placed in a thermostatic water bath. The temperature coefficient of the sensitized reaction was found to be large enough to require careful control of the temperature at which the reaction with dichloramine T takes place. This is best accomplished by means of a thermostatic bath controlled to within at least 0.1° C., preferably in the range of 25° to 28° C. One milliliter of 0.1% dichloramine T in carbon tetrachloride is added and exactly 20 minutes later 4 drops of saturated potassium iodide solution and 4 drops of glacial acetic acid are added with shaking. The iodine liberated by the unreacted dichloramine T is titrated with 0.01 *M* sodium thiosulfate to a colorless end point. The difference in amount of thiosulfate required by the blank and that required when various known amounts of mustard are present gives a measure of the quantity of mustard present. In the range of 0.2 to 5.0 micrograms of mustard the difference in amount of thiosulfate was 0.2 ml. of 0.01 *M* solution per microgram of mustard. There was a straight-line relationship of thiosulfate to mustard. In the range of 0.2 to 1.0 microgram of mustard the average absolute deviation from the mean for ten individual titrations was equivalent to 0.03 microgram.

The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

A Source of Error in the Gutzeit Method for Arsenic

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Department of Health, City of New York, N. Y.

By using a constant-area zinc pellet for the generation of gases, slightly modifying the method of sensitizing the absorption strips, and yet maintaining the simplicity of the Gutzeit procedure, the error is reduced.

IN THE microdetermination of arsenic the Gutzeit method continues to maintain its popularity over other procedures despite the rather poor opinion of its accuracy held by analytical chemists. The reason for this popularity lies in the simplicity of both the procedure and the apparatus required. Investigators (*10*, *21*) generally concluded that the requirements of microchemical accuracy are wanting and that relatively consistent results can be achieved only after considerable experience with the procedure. In attempts to reduce the error the principal modifications proposed were:

Substitution of paper disks (*19*, *22*) or cotton threads (*3*, *4*, *15*, *16*) for paper absorption strips.
Use of activators or zinc alloys (*12*, *15*) for the generation of gases.
Temperature control on whole or part of the generator (*11*, *14*, *15*).
Applications of the molybdenum blue reaction (*9*, *17*, *20*, *24*).
Absorption of arsine into a liquid followed by a titration procedure (*8*).
Spot-filtration of arsine under vacuum (*22*).

None of these variations has achieved general acceptance, because they require the use of special apparatus or the procedure is too complicated. The method of Cassil and Wichmann produces a satisfactory degree of accuracy and shortens the time required for an analysis, but special apparatus and a supplementary iodine titration are necessary. The molybdenum blue procedures and the spot-filtration modification are subject to similar limitations, and How in his searching investigation prescribed a set of carefully standardized conditions in addition to special apparatus which might be acceptable to a laboratory where arsenic analysis is a day by day routine, but would not be adopted readily by the analyst who makes an occasional arsenic determination. Furthermore, the evaluation of these variations by collaborative effort (*6*, *7*, *18*, *23*) has not been completed and hence the official method (*1*) still enjoys considerable vogue.

Since the Gutzeit method is empirical, consistent results depend on close adherence to uniformity in reagents, apparatus, and procedure, rigid specifications for which are prescribed by the official method in all particulars save one—the zinc used for generation of gases. Where stick zinc is used the analyst is instructed to sort out the overactive or underactive pieces on the basis of visual observation of the rate of gas evolution during activation, and to equalize the surface area exposed as far as possible. Cassil (*5*) has shown that the principal factors influencing the length and intensity of the stain are the rate of evolution of hydrogen and the method of impregnating the strips. He found that a definite rate of evolution could not be maintained by the use of granular zinc, though the most satisfactory stains were produced with 20-mesh spherical granular zinc. Gross (*13*) obtained a substantially greater recovery of arsenic with stick zinc than with 30-mesh granular zinc under identical conditions.

The present investigation was pursued on the theory that if a zinc pellet could be devised which exposed a constant surface area to the surrounding liquid, a uniform rate of evolution could be maintained and a more constant reproducibility in length and intensity of stain would result. The author devised such a pellet by molding zinc in the form of cylindrical rod of definite cross-sectional area and rendering the cylindrical surface inactive by a coating of wax, leaving the two plane circular ends as generating surfaces.

EXPERIMENTAL

Preparation of Pellets. For molding zinc cylindrical rods 15 × 125 mm. Pyrex test tubes were employed. Since these vary somewhat, a number having identical inside diameters were selected, and tested with a pair of inside calipers. A tube is clamped on a stand in a vertical position and carefully preheated with a Bunsen burner while a sufficient quantity of arsenic-free zinc is being melted in a Pyrex beaker, then filled with the molten zinc. The tube is tapped to eliminate air pockets and the zinc is

allowed to solidify gradually, the flame being played on the upper end, so that this portion remains in the liquid state longest. This precaution prevents the formation of a hollow core due to contraction as the metal solidifies and results in a solid, uniform cylinder. The zinc is allowed to cool and the cylinder removed by breaking the test tube. With a hack saw the rod is cut into suitable lengths (slightly less than the inside diameter of the generating bottle used), the ends are smoothed on an emery wheel, and the pellet is ready for wax coating.

A mixture of three parts of paraffin to one of Acrowax C (Glyco Products Company, Brooklyn, N. Y.) was found most satisfactory for coating. Some white pigment such as magnesium carbonate is rubbed into gum arabic paste, and the plane ends of the pellet are coated with this paste, and allowed to dry. One end of the pellet is dipped into a beaker of molten wax, withdrawn, and allowed to harden; then the operation is repeated on the other end, the entire surface being covered with a layer about 0.16 cm. (1/16 inch) thick. A second dipping may be necessary. The plane ends are scraped free of wax and soaked in water to remove the coating of paste. The pellet is activated with stannous chloride solution as directed in the official method and stored under water to which a drop of hydrochloric acid has been added. For greater uniformity the author preferred to activate with stannous chloride–hydrochloric acid (1 + 7) solution rather than that of the official method, since this concentration prevails in the actual arsenic determination. It is advisable to scrape away with a knife the protruding collar of wax exposed by solution of zinc. A pellet 3.75 cm. (1.5 inches) long serves for about 15 determinations before becoming too short for further use (Figure 1).

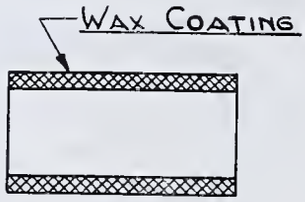

Figure 1. Constant Surface-Area Pellet

Sensitization of Paper Strips. The author has adopted a slight variation in the manner of sensitizing the Hanford-Pratt paper strips.

Instead of sensitizing them each time a determination is to be made, a sheet of 32 strips is cut into 9-cm. lengths and kept permanently suspended in the alcoholic mercuric bromide solution stored in a 10-cc. glass-stoppered graduate cylinder in the dark. Strips are withdrawn as required from this reservoir, pressed immediately between sheets of filter paper, and air-dried for 0.5 hour before using. Length of storage time had no influence on results even after several months of storage. In this manner freshly prepared strips are available whenever required. As a matter of economy determinations were made using strips 3 cm. in length, and to accommodate this variation a constriction was blown into the upper absorption tube, and the strip was dropped into the tube to rest on the constriction. Shorter strips have less tendency to curl, thus producing a more uniform stain (Figure 2).

REAGENTS

Standard Arsenic Solution. Dissolve 1 gram of arsenic trioxide in 25 cc. of 20% sodium hydroxide, saturate the solution with carbon dioxide, and dilute to 1 liter with recently boiled water. Dilute 40 cc. of this solution to 1 liter. Make 50 cc. of the diluted solution to 1 liter; 1 cc. contains 0.002 mg. of arsenic trioxide.

Stock Arsenic Solution 1. To 75 cc. of standard arsenic solution add 150 cc. of hydrochloric acid (specific gravity 1.19), cool, and make to volume of 1050 cc.; 35 cc. of this solution contain 0.005 mg. of arsenic trioxide and 5 cc. of hydrochloric acid.

Stock Arsenic Solution 2. To 150 cc. of standard arsenic solution add 150 cc. of hydrochloric acid (specific gravity 1.19), cool, and make to volume of 1050 cc.; 35 cc. of this solution contain 0.010 mg. of arsenic trioxide and 5 cc. of hydrochloric acid.

Stock Solution of Digested Oysters. Digest a quantity of raw shucked oysters with nitric and sulfuric acids, and treat with ammonium oxalate in the usual manner. Dilute to 500 cc. Determine the acid concentration on an aliquot, then add the exact amount of hydrochloric acid necessary, so that on dilution to 1050 cc. a 35-cc. aliquot contains 5 cc. of acid.

Alcoholic Mercuric Bromide Solution. Dissolve 4 grams of mercuric bromide in 95% alcohol, filter, and add alcohol to make 100 cc.

Potassium Iodide Solution. Dissolve 15 grams of potassium iodide in water and dilute to 100 cc.

Stannous Chloride Solution. Dissolve 40 grams of arsenic-free stannous chloride dehydrate in hydrochloric acid and make up to 100 cc. with acid of the same strength.

For the absorption tower use cleaned sand moistened with 10% lead acetate solution.

PROCEDURE

A series of determinations was made on each stock solution in replicates of six, each set of replicates being run on different dates. Using the same pipet for all determinations 35-cc. aliquots were transferred to generating bottles, 5 cc. of potassium iodide solution and 4 drops of stannous chloride solution were added to each, and the mixtures were allowed to stand for 0.5 hour at 25° C. To ensure uniformity in the amount of stannous chloride solution added, a small pipet was set aside and used solely for this purpose. After introduction of activated zinc pellets, which must lie flat to expose the generating surfaces freely, the absorption tubes were connected, the generators were immersed to within 2.5 cm. (1 inch) of the top in a constant-temperature water bath at 25° C., and the evolution was allowed to proceed for 1.5 hours. The paper strips were then removed and stain lengths measured in the usual manner—by drawing sharp pencil lines across the termination of the stain and averaging both sides of the strip. Readings were made to 0.25 mm.

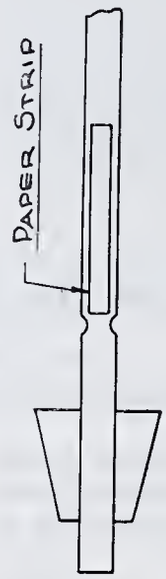

Figure 2. Absorption Tube

DISCUSSION

In the published investigations of the accuracy of the Gutzeit method, Barnes and Murray (*2*), using 30-mesh granulated zinc, reported a probable error ranging from 7 to 100%, covering a concentration range from 40 micrograms of arsenic trioxide down, and Neller, with stick zinc, calculated a probable error of 10% for single determinations. How, using a zinc alloy in stick form, but substituting cotton threads for paper strips and maintaining a very carefully controlled set of conditions, was able to reduce the probable error to 3% for single determinations. The statistical analysis given in Table I presents figures arrived at

Table I. Microdetermination of Arsenic

Trial No.	Length of Stain, Mm.					
	Pellet 1	2	3	4	5	6
	10 Micrograms of As_2O_3					
1	7.0	7.5	7.3	7.8	6.5	7.4
2	7.5	7.6	7.8	8.0	7.4	7.9
3	6.5	7.1	7.1	7.1	7.3	7.1
4	7.4	7.5	7.4	7.6	7.1	7.5
5	7.1	7.4	7.1	7.5	7.6	7.3
Mean = 7.3						
Standard deviation = 0.35						
Coefficient of variation = 4.7 with standard error of 0.61						
	5 Micrograms of As_2O_3					
1	4.5	4.5	5.0	4.5	5.3	5.6
2	4.6	4.9	5.0	5.1	5.2	4.9
3	5.5	5.2	4.9	5.5	5.1	5.2
4	5.4	5.5	6.0	6.0	5.0	5.0
5	5.0	4.9	4.5	5.9	5.0	5.0
6	4.4	4.5	4.8	4.9	5.0	5.0
Mean = 5.1						
Standard deviation = 0.42						
Coefficient of variation = 8.2 with standard error of 0.97						
	Digested Oyster Solution					
1	11.3	12.3	11.5	11.4	11.5	10.5
2	10.3	10.8	12.8	10.1	10.5	11.1
3	9.9	10.6	11.4	9.6	9.8	10.1
4	11.0	9.8	11.8	11.8	10.8	10.3
Mean = 10.9						
Standard deviation = 0.84						
Coefficient of variation = 7.7 with standard error of 1.12						
	Replicates on Diluted Oyster Solution					
5	5.6	4.8	5.4	5.1	5.3	5.5

by employing the more modern concepts prevailing today and is expressed in terms somewhat different from those given above; hence comparison cannot be made at a glance. However, the same statistical method applied to How's results with 10 micrograms of arsenic gives figures which fail to show any significant difference in accuracy compared to those in Table I for the same concentration. Heretofore no attempt has been made at accurate control of either the area of metal exposed to the reaction liquid or the uniformity of activation of the surface. That both these factors have an important influence on the accuracy of the method has been recognized but no means of controlling them has been suggested. The official method directs the analyst to vary the amount of zinc according to the activity of the particular batch used and recommends equalization in so far as possible of the surface area exposed. Left to the judgment of the operator merely on the basis of visual observation, these variables may result in error.

During the course of a determination there is a progressive plating out of metallic tin on the zinc surface, most of which forms a gas-filled sponge which eventually breaks away and floats to the surface of the liquid. The zinc surface retains a thin black coating of amorphous tin uniform in appearance, which does not wash away when a stream of water is played upon it, nor when the pellet is stored under water until again required. The uniformity of this tin plating is an important factor influencing the rate of gas evolution. Measurements showed that the evolution proceeds at a uniform rate and there is no necessity of sorting pellets according to apparent activity. Furthermore, the pellets wear down evenly without pitting or variation in form of the plane surfaces. In the experimental work the six pellets were identified by number, each vertical column of results in Table I having been obtained using the same pellet. No difference in activity of the pellets is discernible. The reduction in error shown in the tabulated results may fairly be ascribed to a control of these variables. It seems likely that a combination of the uniform area pellet and the use of sensitized cotton threads instead of paper strips in the manner described by Cahill and Walters would produce more accurate results in the lower range of 1 microgram of arsenic trioxide. Until a more satisfactory method is developed and generally accepted, approximating the Gutzeit procedure in simplicity, the latter need not be relegated to the limbo of outworn and discarded analytical methods.

SUMMARY

More accurate control of the surface and activation of the zinc pellet in the Gutzeit determination bring about a reduction in error. An improved pellet has been devised which exposes a constant surface area to the surrounding liquid, assuring a more uniform rate of evolution of gases. The pellet serves for a considerable number of successive determinations, and is economical. A more convenient and accurate way of sensitizing and using the paper absorption strips is proposed. These improvements are accomplished without sacrificing the simplicity of the Gutzeit method.

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Volumetric Determination of Water in Paints and Varnishes

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Water can be determined quantitatively in paints and varnishes by a volumetric method with greater accuracy, with small samples, and in less time than by the usual methods.

STANDARD methods of determining water in paints are known to be limited in accuracy and results are reproducible to 0.1% only with difficulty even if 100 grams of sample are used. Whether the determination is made by distillation or by refluxing over Dean and Stark tubes, the water collected is estimated in graduated tubes and suitable apparatus must be erected to test each sample. Time consumed will exceed an hour per sample tested and the amount of water collected is merely estimated. Toluene, xylene, or tetrachloroethylene used for separation of the water prevent wetting of the glass condenser, so that an unestimated quantity of water is always retained and not collected in the graduated part of the apparatus.

The water content of paints and varnishes can be determined accurately and quickly by a simple and efficient technique, employing a specific titrimetric method that involves the use of Karl Fischer reagent (*4*). Results are reproducible to 0.01%.

Karl Fischer reagent for rapid direct determination of water has been successfully applied to oils (*6*), fats (*8*), and waxes (*11*); hydrated salts (*9*); glue, plasticizers, and alcohols (*2*); pulp, paper, cotton, and cellulose (*7*); dry foods (*5*); and liquid petroleum fractions (*1*). With several modifications it can be applied to water analysis of paints and varnishes. The titration assembly is similar to that described by Almy, Griffin, and Wilcox (*2*).

ANALYTICAL PROCEDURE

All apparatus used must be carefully dried. From 10 to 20 grams of paint or varnish are weighed into a 250-ml. glass-stoppered flask. Anhydrous pyridine is added from a buret, the amount varying according to viscosity of sample; 10 ml. may suffice for thinned samples and up to 25 ml. for unthinned samples. The mixture is then swirled thoroughly and 25 to 50 ml. of Karl Fischer reagent are added without delay. The flask is stoppered and placed in an anhydrous glycol bath at 50° C. for 45 minutes. The stopper is loosened once to release pressure, then replaced tightly, and the flask is swirled at 10-minute intervals throughout the warming period. The flask is allowed to cool and contents are titrated electrically by adding pyridine containing 0.1% water.

The titration apparatus consists of a plastic plug equipped with stirrer and tungsten-platinum electrode connected to a Beckman pH meter (model G). Standardization of reagents will complete the operation.

Pyridine (0.1% water), used in back-titrating the unreacted Karl Fischer reagent, may also be used in place of anhydrous pyridine for diluting purposes, and its water content deducted in the calculations. Although anhydrous pyridine is recommended, its possible contamination with water must be checked by the reagent. Anhydrous C.P. pyridine was obtained from J. T. Baker Company, Phillipsburg, N. J., and Karl Fischer reagent was obtained, ready for mixing, from Eimer & Amend, New York.

ACCURACY OF METHOD

Recovery of known quantities of water added to anhydrous paints and varnishes was used to check accuracy. Varnishes and paints found to have no water content or very low known water content were used as a basis and a weighed quantity of 1% solution of water in pyridine was added. Uniformity of solution was obtained by warming at 50° C. for 45 minutes and allowing to cool. Water was then determined on the mixture.

Paint (Anhydrous)	Pyridine (1.16% Water)	Mixture Taken	Water Present	Water Recovered
Grams	*Grams*	*Grams*	%	%
46.1329	10.4001	6.9320	0.2133	0.216
37.9891	13.3605	8.014	0.2419	0.2401
56.4309	30.6477	12.4703	0.509	0.511

A similar procedure was used to compare the method with the present azeotropic distillation method (*3*). An anhydrous alkyd resin of medium oil length was used, 1% water-in-pyridine was added, and aliquot portions were used for each analysis.

Sample Weight Distillation Method	Volumetric Method	Water Present	Water Distillation	Recovered Volumetric
Grams	*Grams*	%	%	%
89.7960	7.4141	1.6477	1.448	1.651
114.3766	4.8950	0.8175	0.699	0.822
97.4304	6.0911	0.4394	0.308	0.435

APPLICABILITY AND INTERFERING SUBSTANCES

Many paint specifications do not specify water tolerances. The volumetric method described has been applied to many types of paints, enamels, and resins, including a number for which water analysis is not required. Resins which in isolated form are known to be insoluble in pyridine are completely compatible when previously dissolved in other solvents. Viscosity of the samples tested varied widely. Dry powdered pigments were checked for interference. Zinc oxide was the only constituent of enamels found to interfere. The presence of zinc oxide did not have to be predetermined, however, since its interference was shown by the behavior of the electrical titration apparatus, in that no state of balance could be obtained and the addition of either Karl Fischer reagent or standard water solution had an unexpected effect on the indicating needle. Thus its presence can be detected by one experienced in handling the titration. However, no paint specifications requiring water analysis have been encountered which also specify zinc oxide pigments, with the exception of Specification T-1715A (*10*), Type I. In the presence of zinc oxide, the A.S.T.M. method for determining water in petroleum products may be used.

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NOTES ON ANALYTICAL PROCEDURES

Precise Low-Pressure Measurements with a Thermocouple Gage

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PRESSURE gages which depend on thermal conductivity (Pirani and thermocouple gages) have been used chiefly for leak-hunting and other rough measurements in the range 0.001 to 0.1 mm. Usually they are operated at either constant current or constant voltage, and in these cases the upper limit of the sensitive range is due not to the thermal conductivity's becoming constant, but rather to the temperature gradient's becoming too small to be measured. The range can be extended considerably by operating at constant temperature (constant resistance on the Pirani gage, constant output on the thermocouple gage). By this means and by using very small dimensions (1-mm. radius), Rittner (*3*) was able to make a Pirani gage sensitive up to 15 mm.

There seems to be no reason, in principle, why these gages should not be used for precise as well as rough measurements. This would be desirable, especially for measurements on condensable vapors where the McLeod gage cannot be used, and also whenever the presence of mercury is undesirable. With the usual setup, the precision is limited by the electrical instruments used. The author has found that when a commercial thermocouple gage is used with sufficiently accurate electrical instruments, and with the addition of a constant-temperature jacket, the precision in the sensitive range is limited only by the pressure measurements used for calibration.

The gage used was a General Electric Type J-1 vacuum gage. It is intended to be used with a constant heater current of 30 ma. and a low-resistance microammeter. Under these conditions, the pressure can be read, at best, to ±4% (at 0.015 mm.); and at 0.1 mm. the sensitivity practically disappears. In these experiments, both the thermal e.m.f. and the heater current were read with a Leeds & Northrup Type 8662 portable potentiometer, the heater current being determined from the voltage drop across a 0.1-ohm precision resistor. In order to permit rapid alternate checks of both quantities, the cold-junction compensating slidewire of the Type 8662 was used as an auxiliary (fixed) potentiometer to compare with the thermal e.m.f. The heater current was adjusted to give a constant thermal e.m.f. of 3.050 mv, (this value is checked occasionally with the main potentiometer. the compensating slidewire being used only as a source of constant e.m.f.); the necessary heater current, which is the measure of pressure, varies from 27 ma. (below 0.001 mm.) to 150 ma. (above 20 mm.). The power supply was a single lead storage cell, with a rheostat consisting of a 100-ohm and a 500-ohm variable resistor in parallel, to give coarse and fine control over a wide range.

To determine the sensitivity, measurements were made on dry air against a McLeod gage and a U-tube mercury manometer

which was read with a cathetometer to ±0.01 mm. The sensitivity may be expressed as the change in heater current for a 1% change in pressure. This was determined by graphical differentiation. When the sensitivity is plotted against the logarithm of the pressure, it falls off symmetrically from a maximum of 0.31 ma. at 0.2 mm., reaching 0.10 ma. at 0.02 and 2 mm., and 0.01 ma. at 0.002 and 20 mm.

In any one run the current could be determined to ±0.01 ma. This means that the gage is sensitive to a 1% change in pressure from 0.002 to 20 mm., while from 0.02 to 2 mm. it is sensitive to 0.1% or less. From one run to another, however, readings varied by as much as 3 ma.. This was attributed to external temperature changes. By attaching a thermocouple to the outside of the gage, it was found that a change of 1° C. in its surface temperature changed the reading about 1 ma. The gage was therefore enclosed in a metal jacket, through which was circulated constant-temperature water. Although the surface temperature was slightly dependent on pressure, it was constant within 0.01° C. at any one pressure.

The major objection to any thermal pressure gage is the curved characteristic which necessitates extensive calibration against some standard. The McLeod gage is not accurate enough to utilize the full sensitivity of the thermocouple gage. Since it appears that a principal use of the gage as a precise instrument would be in measuring the pressure of water vapor above hydrates, a convenient standard is the vapor pressure of ice, which is known (*2*) to ±0.2%. Although a complete calibration curve has not been made, enough points have been measured to show that this is feasible.

The ice was contained in a tube lagged by an oil jacket and immersed in an alcohol bath whose temperature was controlled by a resistance-thermometer type of regulator, operating a pump which circulated alcohol from a dry-ice bath. The temperature in the tube, measured by a three-junction copper-constantan thermopile, was thus kept constant to ±0.02° C., which determines the pressure within 0.2%, the precision of the published data. As much as 1 hour may be required to reach equilibrium. The fluctuations in the gage reading are slightly larger than with air, about 0.02 ma. This may be due to thermal fluctuations, which might be expected to amount to as much as 0.06 ma. in the most sensitive region, but are largely smoothed out by the slow response.

The following readings, made in succession, show the reproducibility of the results whether equilibrium is approached from above or below:

Temperature, ° C.	Pressure, Mm.	Current, Ma.
−45.73	0.0497	60.98 ± 0.01
−36.69	0.1399	86.42 ± 0.02
−23.94	0.529	115.11 ± 0.02
−36.69	0.1399	86.42 ± 0.02

There seems to be no reason why a Pirani gage operated at constant temperature and with sufficiently accurate electrical instruments should not be as precise as the thermocouple gage. However, a metal filament should be used. The author has found that carbon filament bulbs, recommended by De Vries (*1*), are excessively difficult to outgas. Although such a gage is sensitive to ±0.1% in the range 0.1 to 0.5 mm., duplicate runs on water vapor agreed only to 1%, no doubt because of residual air.

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Determination of Gold with Ascorbic Acid

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ASCORBIC acid or vitamin C was isolated by Szent-Gyorgyi (*4*) and its synthesis was later effected by Reichstein (*2*) and Haworth and co-workers (*1*). Haworth and Hirst assigned the accepted structural formula which includes the endiol group. The reducing property of this group is shown in the reduction of gold chloride as represented by the following equation:

$$2AuCl_3 + 3C_6H_8O_6 \longrightarrow 2Au + 3C_6H_6O_6 + 6HCl$$

The detailed investigation of this reaction in the authors' laboratory (*3*) proved that ascorbic acid could be used for the quantitative determination of gold. The present report describes the procedure recommended.

REAGENTS USED. Hydrochloric acid, density 1.19. Ascorbic acid, strength, 4 grams per 100 ml. of solution. Gold solutions, prepared from pure gold as determined by sulfur dioxide method.

PROCEDURE. Dissolve 0.3 gram or less of gold in aqua regia and remove the nitric acid, nitrites, etc., by three evaporations with hydrochloric acid. Dissolve the residue in 3 to 5 ml. of concentrated hydrochloric acid and dilute to 20 ml. with water. Heat the solution to 80° to 90° C., add 10 ml. of the freshly prepared ascorbic acid reagent, and continue the heating for 5 minutes. After cooling to room temperature, filter the precipitate by a porcelain filter crucible, wash with hydrochloric acid solution, 1 to 99, and ignite.

The results are included in Table I. The influence of copper was investigated and the results in Table I prove that there is no interference.

Table I. Determination of Gold

Gold Added *Gram*	Copper Added *Gram*	Gold Found *Gram*	Method
0.2060	...	0.2061	Gravimetric
0.1585	...	0.1586	
0.0969	...	0.0968	
0.0152	...	0.0152	
0.2030	0.1080	0.2032	
0.1215	0.1208	0.1217	
0.0949	0.1930	0.0948	
0.0578	0.0281	0.0580	
0.1403	...	0.1401	Volumetric
0.1051	...	0.1050	
0.0947	...	0.0949	
0.0653	...	0.0653	

VOLUMETRIC DETERMINATION

Gold may be determined volumetrically with ascorbic acid by adding excess reagent to the cold solution of gold and subsequently titrating the excess with iodine. The reaction of ascorbic acid and iodine is described by the following equation:

$$C_6H_8O_6 + I_2 \longrightarrow C_6H_6O_6 + 2HI$$

PROCEDURE. Free the gold solution from oxidizing reagents, add 2 to 5 ml. of hydrochloric acid reagent, and dilute with water to 20 ml. Add 10 ml. of ascorbic acid solution with constant stirring for 5 minutes, then 2 ml. of starch solution, and titrate with 0.1 *N* iodine solution. Blank determinations are required. Some of the results obtained are recorded in Table I.

CONCLUSION

Gold may be determined with excellent accuracy by reduction of gold chloride with ascorbic acid. Copper does not interfere. Gold can also be determined volumetrically with ascorbic acid.

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Preparation of Standard Powders for Reference in Particle-Size Measurement

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IN TESTING new methods and techniques of particle-size measurement and in checking the performance of individual units of apparatus for such measurement, it is often desirable to have one or more standard powders as reference samples. The ideal standard must be composed of particles that possess such a degree of uniformity of size and regularity of shape as to facilitate calibration by microscopic measurement.

The difficulty of obtaining such samples economically and in generous quantity has been a handicap; usually each laboratory has had to make its own. Apparatus and procedures for making microscopic spheres of glass have been described, for example, by Sklarew (*2*), Sollner (*3*), and Bloomquist and Clark (*1*). The steps involved constitute a complete manufacturing process, from massive glass starting material (such as bottles or other macroscopic objects) to finished and classified particles.

It has been found in this laboratory that two types of uniform-particle powders can be prepared from inexpensive commercially available materials, by a small amount of processing with common laboratory equipment.

The first type is spherical, and the coarser of the two. The glass beads used first for coating projection screens, and later also in place of sand for hour glasses, contain a considerable percentage of material, easily separated by sieves, that is close to 100 microns in diameter. A sample investigated (obtained from Seed Filter Co., 47 East Merrick Road, Freeport, N. Y.) was almost entirely (99 to 100%) between the 210- and 74-micron sieves by dry test. The fraction between the 125- and 105-micron sieves (U. S. Nos. 120 and 140, respectively) constituted approximately 13% of the original sample. Most of the particles are accurately spherical, as shown by microscopic examination on a slide coated with grease to prevent rolling and selective orientation.

The few nonspherical particles—irregular fragments, dumbbell forms made by spherical twins, etc.—are removable by either of two treatments. In one method, similar to that used by Sullivan and Hertel (*4*), the spheres are rolled down a vibrating plane inclined slightly to the horizontal, the nonspherical particles being sorted out through their tendency to stall on the slope. More than one traversal of the plane may be necessary. A quicker method, and one that is thorough enough for the purpose, is to pass the spheres through a slightly oversize sieve with little or no agitation of the sieve. In this way a satisfactory refinement of the U. S. Nos. 120-140 fraction was effected with the No. 100 screen.

The second type of powder is rod-shaped, and finer than the first. It is made by pulverizing glass wool. A popular grade of laboratory wool is highly uniform, with a cross-section diameter around 8 microns. This wool can be ground in a laboratory hammer mill, if the skeins are first chopped into short pieces with shears or a print trimmer. A single run through the mill is likely to give insufficient grinding, but quick microscopic inspections, one after each run, will show when to stop grinding. Pebble-mill grinding was tried also, but without success; the wool was always either too little affected or broken into too short fragments. The hammer-mill process, on the other hand, gave a satisfactory range of particle lengths, mainly between 30 and 500 microns.

The range of lengths need not be very narrow, for two reasons: (1) In calculation of the average diameter, either of a single particle or of a collection of particles, if the highly important and much used surface mean is the average that is sought, the average is less affected by variation in the length than by variation in either of the other dimensions of the needle. (With uniform cross-section diameter and variable length, the surface-mean diameter is always between 1 and $1^1/_2$ times the cross-section diameter.) (2) Whatever type of average is desired, it is only in the cross-section diameter that a high degree of uniformity is really necessary, for the length is the dimension most easily and accurately measurable with the microscope.

The hammer-mill product is likely to be discolored somewhat if the interior parts of the mill are of the usual iron alloys, but the color, if objectionable, may be removed by washing with dilute hydrochloric acid.

Final calibration of the sample, in either type of powder, is done with a microscope. In the examples given, the acicular powder will have a surface mean particle diameter around 11 microns; the spherical powder about ten times this value, or 110 microns. The former value is a suitable magnitude for direct reading in air-permeation instruments commonly used for testing of subsieve powders. The coarser powder can be used in the same instruments if provision exists for loading several times the normal amount of sample into the regular sample cell, in order to bring the resistance of the powder column within the range normally encountered with subsieve materials.

The material costs are approximately the following: For the spherical powder, $1 per pound of original beads; if only the cited fraction is utilized, $8 per pound of finished standard powder. For the acicular powder, $3 per pound. It is estimated that a laboratory technician could prepare a pound of either finished powder in 1 to 2 days.

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BOOK REVIEW

Reagent Chemicals and Standards. *Joseph Rosin.* 2nd edition. 542 pages. D. Van Nostrand Co., Inc., 250 Fourth Ave., New York, N. Y., 1946. Price, $7.50.

The present revision of this well-known text includes 52 reagents not in the first edition. Among the additions are: aluminum oxide for chromatography, asbestos (acid washed), cholesterol, dioxane, *o*-phenanthroline, selenium, silver iodate, thiourea, and several of the more important amino acids—arginine, leucine, methionine, tryptophane, etc.

Methods of testing and the limits for impurities have been revised in accordance with the newer, more accurate and precise procedures developed in the past ten years.

All who are interested in the production and testing of reagent chemicals will find the new edition a necessary addition to their working tools.

W. D. COLLINS

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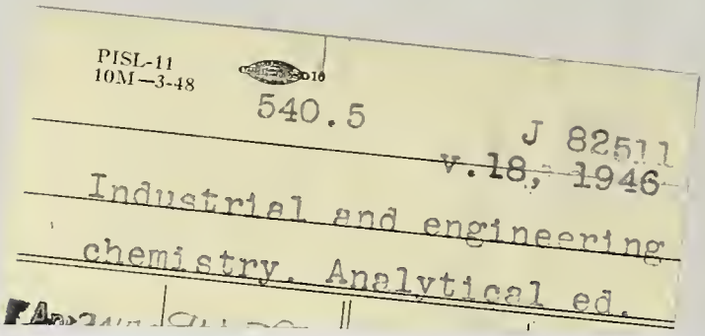
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